NATURE Volume 242
MARCH 2 1973

How Not to Plan for the Future

THE Conservative government is having bad luck with its attempts to make important decisions about important planning questions. For months now, it has been snarled in endless arguments about the rights and wrongs of the Roskill Commission, the *ad hoc* organization which eventually recommended that the third London Airport should be at Cublington, not Stansted (see *Nature*, **228**, 1241 ; 1970), and whose advice had eventually to be overridden in favour of a deserted stretch of mud in the Thames estuary. Last week, Mr Geoffrey Ripon, Secretary of State at the Department of the Environment, found himself having to decide whether or not to accept the recommendations of a similarly ambitious planning inquiry—the Layfield examination of the Greater London Council's proposals for the development of London—and having to decide that very little profit could be derived from a process of inquiry the report of which boasts—and it is the only boast that can be sustained—that it has been the most expensive so far. The Layfield report is the outcome of a year's hard work by half a dozen distinguished people. The cost is estimated to be in excess of a million pounds. The report and the appendices thereto, mostly a list of documents which diligent readers could procure at further cost, is on sale in shoddy mimeograph for £10. For practical purposes, nothing that it says is convincing. Not merely is it fair to say that the inquiry need never have been held but that this embarrassing outcome raises all kinds of questions about the ways in which governments like the British set out to determine crucial issues of public policy concerned with amenity.

The Greater London Council, itself a newcomer on the scene and a successor to the London County Council of the early 1960s, has been an entirely suitable fall guy for the Layfield exercise. The special importance of London as an international centre as well as a metropolis has necessarily directed special attention to the local authority's proposals for the definition of a planning strategy. In December 1969, the Labour government agreed that special arrangements should be made for a public inquiry into the Greater London Development Plan which the Greater London Council had been required, in 1963, to draw up and which it eventually submitted six months late in August 1969. Few will be surprised that the Greater London Council's view of the future was little better than a crude extrapolation of the past. The council originally put forward what amounts to a passive acceptance of recent trends—the tendency of people living in the inner city to emigrate to the suburbs and their tendency then to travel to work by automobile and their unwillingness to rely on a public transport system geared to move lots of people at peak hours but also to be an inconvenient way of getting about at other times. In 1969, in other words, the Greater London Council was apparently happy with the notion that the city centre should continue to lose people and was most exercised to discover some means of enabling them to travel diurnally to and from their work. The result was that the Greater London Development Plan consisted chiefly of proposals for building roads where people used to live and only after the inquiry began did the GLC, repentance for irrationality less evident than a concern for rateable values, suggest that its proposals should be amended so as to encourage a less rapid drift of people from the city.

Those who believe that sportsmen should not shoot sitting ducks will despise what the Layfield report has to say about the Greater London Development Plan. The august and hard-working committee of inquiry is right to say that the plan is over-ambitious—to pretend that it is possible to predict for several decades ahead what will happen to the movement of population or the disposition of industry is a little like trying to predict what will be the next great innovation in technology—the trouble is that the hapless Greater London Council was required to do no less by the Town and Country Planning Act of 1947 and 1968. It is also easy to say that the plan was inconsistent—for better or for worse, British governments have found it necessary to agree that the Greater London area should be a single planning unit but that much smaller parts of this vast concentration of people, the London boroughs and the outlying counties, for example, should be politically autonomous. Is it any wonder that a planning authority should find itself unable to reconcile the conflicting interests of its constituents with its own grand vision of the future?

The Layfield committee has great fun with the Greater London Council's failure "to relate information to policies", but that is not surprising. The committee says that "one of the most notable features of the Greater London Development Plan is the independence of the policies in the plan from the facts gathered . . ." and it says that "in many cases we had the greatest difficulty in seeing why the facts led to the solution set forth in the plan . . . and in some cases . . . we never found out at all". What the committee has in mind is the Greater London Council's attachment to ideologies such as the Green Belt or its political awareness of its impotence in the face of the conflicting interests of the Greater London Council's constituents. But might it not have been more cogent, or at least more sporting, to have complained at the planning legislation and not to have taken pot shots at that sitting duck, the Greater London Council? To be sure, it is fair to say that the council has consistently failed to relate its planning policies to its philosophical objectives. But the plain truth must surely be that the council is prevented not merely by its constitution as a federal body, by its existence as an inheritor of previous shibboleths such as the Green Belt and by its awareness that radical proposals might jeopardize its own survival, from being anything but a temporizing institution, ill equipped to face the future but constitutionally unconvinced that there will be one.

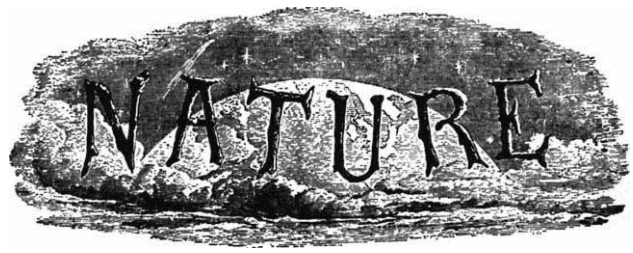
The most famous and notorious part of the original development plan was that proposing three rings of motor-

ways around London intended both to help commuters get to work and traffic happening to pass nearby from travelling through congested parts of London. Understandably, people who live in London have for several years been up in arms at the prospect that their houses will be destroyed to make way for motorways or that their favourite patches of greenery will be despoiled. The Layfield report might have said, but it has not done so, that the Greater London Council has consistently failed to demonstrate that its proposals for building motorways were in some sense or another optimum solutions to a difficult problem and even that the motorways would be socially beneficial in the widest sense, balancing economic benefit against social disbenefit. Innocent readers of the Layfield report will look in vain for the kinds of arguments, inevitably somewhat mathematical or just plain arithmetical, that might have thrown light on this important question. Instead, what they will discover is that Layfield and his men, like conjurers pulling rabbits out of hats, has struck out parts of the motorway system which they consider to be unnecessary, have confirmed that other parts should be built, and, in doing so, have given the impression that the chief defect of the Greater London Development Plan is not its conception but its particular choice from the several somewhat arbitrarily balanced alternatives with which it was presented. To be sure, there are plenty of platitudes about the need to encourage travel by public transport, the modern equivalent of the fondness of the 1950s for motherhood, but the ingredients are much the same.

If, among sportsmen, it is agreed that sitting ducks are not fair targets, so it should be acknowledged that sportsmen should not be shot at while unable to defend themselves, which is why Mr Geoffrey Rippon's commentary on the Layfield terms is also tantamount to taking a mean advantage. He says that there is probably much to be said for the Layfield report's belief that a more rapid reduction in the population of London than the Greater London Council is happy about could easily be stomached but asks whether it can be right that the rate of building in satellite towns should be increased and whether the Layfield committee can be right in thinking that high density development should be avoided but that the council should also take steps to avoid the under-use of land, thus making still more uniform the distribution of people within the Inner London area. The trouble, of course, is that it takes only a minor Solomon to make such a comment. Constitutionally, Mr Rippon and his predecessors invited the Greater London Council to say what they would like, then invited the Layfield committee what it thought of what the Greater London Council had said: so vast are the uncertainties that it needs not a senior cabinet minister but only a schoolboy to say that there are grounds for disbelieving both opinions. It is in exactly this spirit that Mr Rippon makes his own amendments to the proposals for road building in London as originally advocated by the Greater London Council and then amended by the Layfield committee.

What is the moral? First of all, there must now be serious doubts of the wisdom of entrusting professional planners with planning. If wars are too important to be left to the generals, is it sensible that people should allow disinterested professionals to decide how people must live? For the truth is that planning is not and never can be a strictly technical question. It is a political matter, and what the government must decide is not how best to do the local authority's job but rather how to define its own relationship with London planning authorities in such a way that they will be able to do their job efficiently. The worst feature of the whole Layfield episode is not the abysmally unquantitative characteristics of the committee's report but the fact that, having created a planning authority, the government (or, more strictly, its predecessor) decided to do the job itself. The second complaint against this tawdry happening is that none of the three parties to the argument, the Greater London Council, the Layfield committee and the government, has been able to lift its vision above the solutions to familiar problems with which town planners have been wrestling for decades. Why does none of these vast and expensive documents consider other kinds of solution to the problem of living sensibly in cities such as London? In all kinds of ways, it might be better to abandon the Green Belt for the sake of having a city in which people were closely connected together but also with the countryside, possibly in a cellular kind of city. Ultimately, the failure to be imaginative must be blamed on the government, for that is the level to which planning authority has been usurped. Luckily, but ironically, the outcome of the Layfield report is unlikely to be permanent: there is at least a chance that by having approved the plan to build motorways in central London, the government will have so alienated opinion in the city that the Conservative Greater London Council will be defeated by its Labour opponents in May, whereupon the whole issue of the Greater London Development Plan will be put back another decade. This may not be progress, but it is a kind of justice.

100 Years Ago



WHEN is the foundation-stone of our grand new Natural History Museum to be laid? Is Government waiting for the advent of fine weather in order that the ceremony may be as auspicious and imposing as possible? We can hardly believe the current gossip that the fiscal authorities of the country have quietly retired the thousands said to have been voted for the purpose, in order that a saving might be effected in their expenditure, and a handsome surplus be vaunted of in the forthcoming budget. Meanwhile see what our young, energetic, long-headed cousins on the other side are doing. A new Natural History Museum is about to be erected in New York 800 feet long by 600 wide, which will be the largest building in America. 100,000*l.* was voted last winter by the legislature to commence it, and 200 men are already blasting for its foundations. It is eventually to cost 2,000,000*l.* sterling, and fifteen years will be occupied in its construction. This great building is to cover fifteen acres of ground, and is to be situated on Montallan Square, facing Eighth Avenue and Central Park. The front portion is to be finished directly, and the back portion is to be finished from time to time as needed, and as appropriations are made for it. The material is to be granite.

From Nature, 7, 349, *March* 6, 1873.

OLD WORLD

Open University and Fourth Television Channel

A FOURTH television channel devoted to education would find favour with the Open University. In the second report of the Vice-Chancellor, published today, Dr Walter Perry says that an education channel which would combine the schools programmes and the further education programmes of the BBC and the IBA with the Open University broadcasts would be "a splendid combination" because the schools programmes and those of the Open University would be broadcast at different times of the day.

Dr Perry said, this week, that there are strong reasons for a fourth television channel for education but that there are enormous political, economic and technical problems to be solved first. But, said Dr Perry, a more practical proposition at present would be the setting up of an educational radio channel.

During 1972, the Open University took up 527 hours of television time compared with about 360 hours devoted to schools TV and 310 hours for further educational programmes on the BBC. The Independent Broadcasting Authority, on the other hand, devotes at least nine hours a week during the school year to school programmes and three hours a week for fifty weeks a year to adult educational programmes. So in all, more than 1,600 hours on all three television channels were devoted to education during 1972.

The Open University calculates that it will need 37 hours a week of television time in 1976—but its present contract with the BBC, which expires in 1976, is for 30 hours. If the university does go on the air for the longer time during the entire university year of 36 weeks, then it will increase the air time from 527 hours to over 1,250 hours.

The total number of hours for education will still, however, be much less than the 6,800 hours a year needed for a full television channel, even if schools and further education programmes are increased drastically.

During 1972 the Open University used up 383 hours of radio time compared with 457 hours for school broadcasts and 354 hours for further education broadcasts. In 1976 the university reckons that it will need more than 1,000 hours of radio time.

But there seems to be little chance of a further television channel being set up before 1976 when the BBC's charter expires and when the independent television companies' contracts run out. A recent question in the House of Commons which inquired whether the Minister of Posts and Telecommunications would authorize the setting up of a second independent television channel was answered with a straight "No" by Sir John Eden. In his report Dr Perry says that the expertise of academics on television has astounded many people and "some old and well-revered shibboleths have been knocked down". In a wider context Dr Perry says that it is more than likely that the educational methods pioneered by the Open University will prove to be of great use "in the larger world outside the university".

As yet, however, the teams making the programmes have not evolved any radically different techniques, but as the teams gain experience of the needs of students such techniques will develop. In particular Dr Perry sees radio vision being used to a greater extent in the future. Radio vision, which is basically a series of colour slides accompanied by a radio broadcast, is not second best television or glorified radio, says Dr Perry, but a distinct technique with quite definite and specific advantages in its own right.

During 1971 the Open University enrolled 20,000 students at a total direct cost of £2.2 million. The gross income for the year was £7.02 million, of which £6.02 million was granted by the Department of Education and Science, and £0.89 million came from student fees. The remaining £0.11 million was obtained from short-term investments. Sixty-nine per cent of the income was spent on fixed overheads and the remaining £2.2 million on direct student costs. Nearly half the sum spent directly on students went to pay tutors and counsellors—both full and part time, and the remainder was distributed between the cost of home

SELECT COMMITTEE

Hovertrain Demise is Aired Again

THE Select Committee on Science and Technology spent a morning last week trying to nail the blame for its own ignorance of the government's decision to cancel the hovertrain at the door of either British Rail or the National Research Development Corporation (NRDC).

Both organizations knew of the government's decision to cancel Tracked Hovercraft Limited's work when they wrote the memoranda for the Select Committee on the hovertrain in early February. Neither revealed the government's decision, which was not announced until Mr Heseltine gave evidence to the committee on February 14.

The select committee wanted to know why neither body had passed its information on. Mr Richard Marsh, chairman of British Rail, said that it was not BR's job to reveal government decisions. He also pointed out that the select committee had not asked him if the project was to be cancelled. When asked if Mr Heseltine had asked him to keep quiet about the decision, Mr Marsh replied "quite categorically no".

NRDC knew that the decision to cancel would be taken as early as January 23. Its reason for not telling the select committee was that Sir Frank Schon, NRDC's chairman, had made an agreement with Mr Heseltine not to reveal the decision. Sir Frank had been told of the impending decision on January 23 because an NRDC board meeting on January 24 had to decide whether to proceed with the project. "I insisted that (the decision to scrap the project) was classified information", Sir Frank said.

Mr Richard Marsh, in his evidence, defended the Advanced Passenger Train against allegations made about it last week by the staff association of Tracked Hovercraft Limited. It was not true, he said, that APT had made two unsuccessful runs; it had made one successful one. It is not being significantly rebuilt, as alleged; the gas turbines have been replaced by an electrical propulsion system in the prototype because that had always been planned, not because of problems with the turbines (APTs will be powered by both electric traction and gas turbines when they come into service). The allegations were "totally inaccurate and untrue", Mr Marsh said, "they are wrong, irresponsible and very damaging to our interests".

Mr Marsh emphasized that there was never any competition for government funds between Tracked Hovercraft and British Rail, "at no point have I heard this suggested". But he did say that the cancellation was "one of the few times a government has cancelled (a project) without wasting a great deal of public money". But the parts of the system that had potential had been rescued.

experiment kits, summer schools and printed materials.

The BBC was paid £1.47 million for making programmes which amounted to more than 30 per cent of the fixed overhead expenditure of the university in 1971. At present the Open University programmes are prepared at Alexander Palace, but by 1976 the university hopes that the programmes which it needs will be made at a new audio visual complex to be built on the university campus at Bletchley. The Department of Education and Science has agreed in principle that the complex can be built—to be staffed and manned by the BBC—but at present the university and the DES are negotiating the money to be allocated. This, in the long run, will determine the number of studios to be built, but to build and equip a viable complex will cost about £4 million according to the Open University.

SWINE DISEASE

Control of Swill Plants

A MINISTERIAL order to control the operations of pig swill plants is to be introduced shortly. Fears are growing that unless steps are taken soon the virus that is causing the current epidemic of swine vesicular disease may be recycled through pig swill.

The movement controls imposed last week on pigs throughout England and Wales should do much to limit the spread of the disease—more than 40 out of the 55 cases that have followed the original outbreaks have been attributable to stock movement—but at least five cases may be the result of contaminated swills and the original outbreaks were all on swill-fed farms.

Recycling can occur if a pig is slaughtered while it is incubating the disease (incubation takes 4 to 6 days). Those parts of the animal not used for human consumption go to plants which manufacture pig swill for use on more than 5,000 farms in England and Wales. If the meat from the slaughtered pig is not properly sterilized the virus will be carried over into the swill resulting in further infection.

This may not be the real problem, however. Swill plants are supervised by local authorities, and ministry officials are fairly happy that the meat is being sterilized. The problem chiefly lies in the organization of the plant. Sterilized swill may be recontaminated during the handling of raw swill, and it is this aspect of the operation that ministry officials want tightened up.

Swine vesicular disease is in itself not particularly serious. Infected pigs can recover. The problem is that the symptoms are identical to those of foot and mouth, and if swine vesicular disease was allowed to become endemic in Britain it could mask the much more serious foot and mouth disease. Foot and mouth can kill large numbers of cattle and pigs, and survivors give poor milk and meat yields. The only way to safeguard against this is to stamp out swine disease.

Not that that is proving easy. Although the amounts of virus excreted by infected animals are low compared to the amounts emitted by foot and mouth victims, the swine disease virus is remarkably persistent. There is no question of it being an unbeatable virus, but two of the three farms that were restocked recently have had fresh outbreaks. As a result restocking is not being attempted until eight weeks after disinfection of the farm is complete. Fortunately airborne transfer of the disease is unlikely as the animals excrete only small quantities of virus, and the chief cause of the fifty-five secondary cases has been movement. Twenty herds contracted the disease at markets, five herds were infected by the transfer of infected pigs onto the same farm, and seventeen got the disease from infected lorries. Theoretically lorries are disinfected between each load of livestock, but clearly this is not being done or is not being done efficiently. Greater vigilance and the ban of stock movements should help. Of the remaining cases, one is due to local spread—by vermin or birds—five cannot be easily accounted for, and a further five may be the result of the virus being recycled in swill, although there is no positive evidence that this is so.

Contaminated swill may have been the cause of the original outbreaks. Poland, Austria, France and Italy all had outbreaks of the disease late last year. Austria almost certainly imported it from Poland. But Poland, France and Italy all import pork from the far east and it is possible that the disease came to Europe that way. Britain too imports pork, although not directly from the east, and it is possible that contaminated carcasses were imported, and their offal used as swill on the five farms were the primary outbreaks occurred.

But whatever the cause, the disease still has to be controlled. The movement ban and swill controls should solve the problem in time, but the persistence of the virus could mean that it will be quite a long time. To date more than 27,000 pigs have been slaughtered, but slaughter seems the only policy. Developing a vaccine to combat the disease would take time, and the rapid turn round on pig farms (the average life of a pig in Britain is a little over five months) would make administering it an almost impossible task.

Lord Snow to the Fore

SPEAKING at the Annual Dinner of the Institution of Electrical Engineers last week, Lord Snow described plans to shift the balance in universities towards the social sciences as "passing the confines of lunacy".

He went on to say that "we shall be spending thousands of pounds educating young people in social sciences and I cannot imagine any more foolish waste of money". He also bemoaned the fact that engineers in this country are for some reason less respected than in other countries.

Lord Snow said that far from being encouraged to study social sciences, many more undergraduates should be doing hard subjects like mathematics. They could acquaint themselves with social sciences at a later stage, he contended, as people had managed to do quite successfully in the past.

ISAAC NEWTON TELESCOPE

To Move or Not to Move

THE row over the wisdom, or otherwise, of siting the 98-inch Isaac Newton telescope above the Pevensey marshes of Sussex has surfaced at last in the correspondence columns of *The Times*. Many astronomers have been unhappy about the location for years, and criticism has reached a high pitch since the inauguration of the telescope five years ago, but for the most part astronomers have succeeded in keeping the debate to themselves. This self-imposed embargo has now been breached by Mr W. Bates of Cheltenham, who wrote to *The Times* in response to an article by their science correspondent reporting the view of the Director of the Royal Greenwich Observatory, Dr Margaret Burbidge, that the telescope cannot be properly used at its present site, and that ultimately it will have to be moved. "Who," Mr Bates asked, "was responsible for this disgraceful waste of public money? What action is being taken against them?" (*The Times*, January 31, 1973.)

So far Mr Bates has not had his answer. But Mr P. Lancaster Brown of Beaconsfield, who has written several articles about the telescope, including one commissioned by the Central Office of Information, wrote to give some facts about the history of the telescope (*The Times*, February 5, 1973). The telescope was first suggested by Professor H. H. Plaskett in 1946 during his presidential address to the Royal Astronomical Society, and the original design was a Schmidt with an aperture

between 49 and 72 inches. The abandonment of this proposal, which some astronomers still regret, came about, according to Mr Lancaster Brown, because some members of the board of management of the project felt that this design would be merely duplicating the Schmidt telescope on Palomar Mountain, California. The telescope that was finally built was a Cassegrain reflector making use of the 98-inch blank presented by the MacGregor Trust of Michigan.

According to Mr Lancaster Brown, the selection of the site was justified on the grounds that the number of clear nights in Britain is not hopelessly inferior to that at the sites of other big telescopes, and he cites the following figures for the number of clear nights per year at Herstmonceux (the site of the Isaac Newton telescope) and two other observatories: Herstmonceux, 1,900 hours; Mount Wilson (California), 2,700 hours; Dominion Observatory (British Columbia), 1,247 hours.

Mr Lancaster Brown says that "in hindsight it seems that the final design choice was mistaken, and that the original Schmidt telescope suggested by Professor Plaskett would have provided a more suitable instrument", but he disputes the view that the construction of the telescope was a disgraceful waste of public money. The question which should really be asked, he suggests, is "Was the meteorological data which influenced the decision to place a large telescope in Sussex wrong"? And he says it would be interesting to compare the number of clear or partially clear nights at the telescope with the number of nights that the instrument has been used by the incumbent staff.

Professor R. A. Lyttleton of St John's College, Cambridge, last week jumped in with a theoretician's view of the debate (*The Times*, February 24, 1973). He says that the site was chosen for reasons of prestige, even though its unsuitability was known at the time, and that dissenters were warned that any questioning about the site might lead to the cancellation of the project.

Professor Lyttleton goes on to argue that in any case most of the material in the universe is out of sight, so that astronomy is almost wholly a theoretical subject. The money spent on the telescope was enough to set up permanent posts in theoretical astronomy in almost every department of mathematics in Britain.

According to Professor M. J. Seaton of University College, London (*The Times*, February 27, 1973), Professor Lyttleton's views on the pre-eminence of theoretical astronomy "leads to the most arid scholasticism" (although, to be fair, Professor Lyttleton's letter can also be read as making the point that observational astronomy should be left to those countries with the right climate). Professor Seaton goes on to say that one of the arguments for siting the telescope in Britain was to give young astronomers an opportunity to learn to use a large telescope, and to develop new techniques of instrumentation. "No one who has studied the problem carefully seriously suggests that the INT should be moved; the decision has been made, the telescope performs a useful function where it is, and the cost of moving it would not be much less than the cost of an entirely new telescope."

Mr Bates, who started the correspondence, would be justified in complaining that none of the letters answers the points he raised. Professor Seaton notwithstanding, many astronomers would like to see the telescope moved to a more congenial site, and no doubt there will be an interest in the calculations which Professor Seaton says militates against such a move. With there now being talk about the establishment of a new British observatory in the Mediterranean area, possibly on the Spanish island of Tenerife, astronomers will now be wondering whether the arguments which prevented an overseas location for the Isaac Newton telescope will be voiced again. It may well be that at present the greatest obstacle to the establishment of the new observatory is Britain's dispute with Spain over Gibraltar.

INDUSTRIAL INNOVATION

CSII Runs into Hard Times

THE activities of the Centre for the Study of Industrial Innovation have been suspended. Lack of funds and the refusal of British industry to support it have left the centre with little hope of continuing its work except under the wing of a university.

George Teeling-Smith, the centre's director, is "pretty disillusioned about the whole thing". The centre only costs £20,000 a year to run, part of which it earns through contract research, and the simple fact is that industry has refused to help although often paying lip service to the idea.

The centre's financial crisis has been looming for some time (see *Nature*, **238**, 365; 1972). By the end of last year it looked as though the money was not going to materialize and Mr R. Jones, the CSII's resident economist, decided to take a job in industry. The centre aborted its study on the effect of government purchasing on innovation as it was nowhere near finished, and went into hibernation. A few weeks later, but too late in the day, the Social Science Research Council approved a £5,000–£6,000 grant to the centre to study the effects of standardization on innovation.

The centre's best chance of survival lies in cooperating with a university. Both Aston University and the Cranfield Institute of Technology have shown interest. Dr A. H. Chilver, Cranfield's vice-chancellor, said this week that he has discussed the centre's problems with George Teeling-Smith and Edward Hawthorne, one of its trustees, and is eager to define precisely what the centre wants to do and then see if there are ways in which its work could match Cranfield's. "We have no intention of taking the centre over" he said. "We want to see if we can play some part in continuing and developing its work."

If Cranfield does help rescue the centre from its current predicament, funding will almost certainly come from outside the normal run of university sources, possibly from the research foundations. Discussions on finance will take place in the next few weeks. If a link is formed the centre will probably move to Cranfield. "It would probably be important for it to live in an advanced technological environment," Dr Chilver said, "and it would be a logical move to bring it to Cranfield."

Any development resulting from a link with Cranfield would please George Teeling-Smith. "I don't think a relatively small organization will significantly alter the climate for innovation," he says. "British industry needs an atomic bomb under it rather than the squib that we were providing."

Having spent three years being greeted with welcoming smiles but empty handshakes, Teeling-Smith is "a bit sick of the attitude of British industry as a whole". The pharmaceutical companies would have financed the centre (they provided the initial capital), but they already pay for the Office of Health Economics which runs similar studies on health problems, and a similar organization under a different name seemed pointless. The National Economic Development Council (NEDC) contributed, the National Research Development Corporation (NRDC) provided a pittance, and half a dozen other companies stumped up the princely sum of £150 each.

In its three years the centre has published seven reports (one of which for NEDC is due out in March), five of which it researched itself. While its work has not been greeted with total critical approval, it has at least stirred a pot that until recently no one was bothering to watch.

NEW WORLD

Energy from Above and Below

by our Washington Correspondent

FOR many people in the United States, the "energy crisis" finally arrived this winter. Schools and offices have been shut down in several parts of the country because of shortages of oil, and there are now dire warnings of petrol rationing in some areas in the spring. Although there are good reasons to suggest that such acute shortages of oil are chiefly a product of poor distribution and lack of planning and that they are also a direct consequence of the fact that energy is still so cheap in the United States that it is squandered, the energy crisis has triggered a great debate about future supplies and uses of power. Indeed, President Nixon will soon send a message to Congress outlining his Administration's plans for dealing with fuel shortages, and Congress itself is busy forming committees to look into the problems. One result has been an awakening of interest in unconventional sources of energy.

Nobody is suggesting that new sources of energy will help alleviate the present difficulties, but there is hope that they may make a significant contribution to energy supplies by the end of the century. In particular, the Sun and the interior of the Earth are being touted as especially promising sources of power, and two reports prepared with funding from the National Science Foundation have added weight to such suggestions.

As far as energy from the Earth's interior is concerned, one report states that "geothermal resources, by approximately 1985, can have a potentially enormous impact in supplying the nation's need for energy". And the other report, which is concerned with solar power, states that "the panel is confident that solar energy can be developed to meet sizable portions of the nation's future energy needs". The catch, however, is that before such rosy predictions can be realized, large expenditures on research and development will be required, and it seems unlikely that they will be forthcoming.

President Nixon's energy message will undoubtedly highlight the fact that the budget proposes expenditures of $772 million in 1974 on energy research and development—an increase of $130 million over planned expenditure this year. But nuclear energy is set to carry off more than $560 million, with the lion's share going to the breeder reactor, and the total expenditure on solar and geothermal energy together is set for only $16 million. In contrast, the report on geothermal energy recommends expenditures of $41.7 million next year, and the solar energy report calls for $3,500 million to be spent on developing that resource over the next 15 years. The Administration is clearly not quite so enthusiastic.

The chief architect of the report on geothermal energy is Walter J. Hickel, former Secretary of the Interior in the Nixon Administration. With funding from the National Science Foundation's Research Applied to National Needs (RANN) programme, Hickel called together a conference of some 50 scientists and engineers last year, and the report (*Geothermal Energy*, available from the University of Alaska) is the fruit of that conference. Although Hickel is quick to point out that "we do not see geothermal energy as the only answer to the future, nor necessarily the best answer," his report makes the optimistic prediction that geothermal resources in the United States should be capable of supplying some 1,000 million megawatt hours of electricity by 1985 and 3,100 million by the year 2000. In contrast, the power demand of the whole of New England is now less than 60 million megawatt hours a year.

That estimate rests not only on the assumption that a vigorous research and development programme will be funded, but that such a programme will pinpoint and assess geothermal resources in the United States, that it will result in the technology to develop the resources, and that the energy so produced will be able to compete economically with energy produced by other means. The report candidly admits that "only time and research funding can validate or invalidate" those assumptions, but it is pointed out that they have been subjected to the scrutiny of a large number of knowledgeable scientists and engineers, who have concluded that substantial funding is warranted.

The idea of geothermal energy is essentially to tap the great heat reservoir in the Earth's core and to turn it into electric power. The heat, which is derived from radioactivity in the Earth's core and friction resulting from solar and lunar tides as well as the motions of the crustal plates, can theoretically be tapped simply by drilling through the Earth's crust, but it is practical to exploit the heat reservoir only when it is near the surface. And that means chiefly at the edges of the crustal plates and recent volcanic formations. Since much of the western part of the United States has an abundance of volcanic rocks of recent origin, the "potential geothermal resources appear to be very large", the report suggests.

The resources considered for commercial exploitation consist of deposits of superheated steam which contains little or no liquid water, deposits of hot water and hot, dry rocks. Steam deposits are the easiest to exploit since the steam can be fed almost directly into turbines to generate electricity, but they are few and far between. Five such deposits have been discovered, but only three are considered commercially exploitable—one, at Larderello in Italy, has been used to generate electricity since 1904, and another, in Northern California, has recently been exploited.

Deposits of hot water are much more abundant than deposits of dry steam, but they are more difficult to exploit. If hot enough, the water, which is under great pressure in the deposits, could be flashed to steam and used to drive turbines. Deposits of cooler water, below about 200° C, may be exploited by transferring their heat to a more volatile liquid in a heat exchanger, and the vapour from the second liquid would then be used to drive a turbine. This is one area which the report suggests merits further study.

As for hot rock reservoirs, although they are the most abundant geothermal resources, they are also the most difficult to exploit. Several methods have been suggested, all of which involve sinking two holes, fracturing the rock between them with explosives or hydraulically, and circulating a liquid through the fractured hot rock. The report points out that if the technology for recovering the energy from dry rocks is developed, and "once deep drilling technologies are available, geothermal energy sources might be extended to areas of normal heat flow, thereby producing a truly immense energy source".

Hickel's report recommends that the federal government should spend about $680 million over the next ten years on research and development into the exploitation of geothermal resources, nearly half of which should be devoted to resource exploration and development. Other problems needing atten-

tion, the report suggests, are drilling techniques, methods for assessing the size and deliverability of geothermal resources, binary fluid systems for extracting energy from reservoirs which are too cool to provide steam to drive turbines directly, desalination of brines to extract minerals and to provide fresh water along with energy and on the environmental effects of exploiting geothermal power. So far, federal funding on such problems has been small, and there has been a lack of a defined programme for exploiting geothermal energy.

If the report on the potential for geothermal energy is optimistic, the report dealing with solar energy is no less rosy. Prepared by a panel organized jointly by the National Science Foundation and the National Aeronautics and Space Administration, the report concludes not only that "there are no technical barriers to wide application of solar energy to meet US needs", but also that a "substantial development program" could meet the necessary technical objectives by the year 2020. By that date, the panel believes that solar energy could provide economically up to 35 per cent of total building cooling and heating, 30 per cent of gaseous fuel used in the US, 10 per cent of the liquid fuel and 20 per cent of the electric energy requirements.

All that is needed for solar energy to live up to these predictions is a research and development budget of some $3,520 million over the next 15 years, some technological breakthroughs and the work of market forces which are expected to increase the cost of energy from other sources to make solar power more competitive. In short, the panel suggests that "on close examination, the possibilities for the economic use of solar power, given reasonable R and D support, appear much better than generally realized".

The research and development programme outlined by the panel would concentrate on three chief areas of direct use of solar power—thermal energy for buildings, the production of fuel from organic materials and electric power generation by thermal conversion and by photovoltaic cells—and in addition the panel suggests that wind energy and thermal differences in the oceans could be tapped for electric power generation. Major technical problems remain to be solved in each area, but if the funding is made available, the panel believes that solar power could be used to heat buildings in about 5 years' time and cool them in about 6–10 years, that synthetic fuels could be produced from organic materials in 5–8 years and electricity in 10–15 years.

Residential heating and cooling "has the highest possibility of success", the panel suggests, but there is need for development of more efficient solar heat collectors for use on rooftops, and the efficiency of cooling systems powered by solar heat must be improved. Nevertheless, the costs of developing such a system are reckoned to be about $100 million over 15 years, which is a small fraction of the predicted fuel savings.

One reason why both geothermal power and solar energy are receiving considerable attention in the United States is because they have the backing of environmentalist groups. The energy crisis seems likely to hurt the cause of environmentalists badly, particularly if they are blamed for part of the shortages by blocking nuclear power plants or the Alaska pipeline, and environmentalists are urging research and development on those forms of energy production which are likely to have the least impact on ecological systems.

Solar energy seems to fit that bill, since for applications such as building heating and cooling and electric power generation, little or no waste products are formed, and no resources would be depleted. Geothermal power production, on the other hand, is likely to involve noisy, dirty plants, and there is possible environmental damage from contamination of groundwaters with brines that are reinjected into the soil. Nevertheless, the environmental damage from such power plants would be limited exclusively to the site, since they would use no transported fuel, nor require the disposal of radioactive or other toxic wastes.

Whether the hopes of the environmentalists or the rosy predictions of the two reports on solar and geothermal energy will be realized seem to depend chiefly on research funding and the solution of important engineering problems. The Administration is, however, understandably sinking its money into less technologically uncertain ventures, such as the breeder reactor.

BIOMEDICAL RESEARCH

Medical Ethics Examined

by our Washington Correspondent

THE Senate Health Subcommittee last week held three days of hearings on biomedical research involving human subjects, and it opened up a Pandora's Box of problems involving medical ethics. The hearings ranged over cases of unapproved contraceptive drugs being given to poor people in Tennessee and to university students in the United States, and they also took a look at some of the medical issues involved in the fields of brain research and psychosurgery.

Few questions were answered—that was not the object of the exercise—but with good stage management, human interest, pathos, displays of anger and, of course, the drawing power of

ASTRONOMY

Funds for Poland from the NSF

by our Washington Correspondent

THE National Science Foundation has announced that it will release $1.4 million in Polish currency owned by the United States for construction of a centre for theoretical studies in astrophysics. The centre, which will be built in Warsaw, has been under consideration since 1971, and the announcement of the plans coincides neatly with the current celebrations of the 500th anniversary of the birth of Copernicus.

The money will be spent over three years, as part of the NSF's Special Foreign Currency programme, which uses currency derived chiefly from the sale of agricultural products for scientific purposes. The Polish Academy of Sciences will also put up about $622,000 for furnishing and equipping the centre, and will support its operation. Poland is one of eight countries which benefits from the special currency programme.

The astrophysical centre will consist of laboratories, a lecture room, a computing centre, administrative offices and facilities for visiting scientists, housed in a building with some 56,000 square feet of floorspace. Construction is due to begin in June, and the building should be ready by October, 1975. It will be named the Copernicus Astronomical Center.

In November last year, the governments of the United States and Poland signed a broad agreement on cooperation in science and technology, which included plans for sharing of research data between scientists in the two countries and the exchange of personnel. Although the Copernicus Astronomical Center was being planned well before the agreement was reached, it is the first large project to be announced since the agreement was concluded last year.

Senator Edward Kennedy, the chairman of the subcommittee, they received considerable public attention. The central issue to emerge from the hearings is simply how can the rights of a patient be best protected, without undue interference from the Federal government in the practice of medicine?

The first two days of hearings centred on an apparent loophole in the food and drug laws which allows drugs to be prescribed, sometimes on a large scale, for unapproved uses. When a new drug is placed on clinical trials, the Food and Drug Administration requires an investigator to obtain a permit, his trials are closely monitored, and several precautions are required to ensure that the patient is aware of the risks involved in the trial. The same procedure must also be followed when a drug which has already been approved for one use is tested for a different application, but since that drug will already be in the pharmacies, there is nothing to prevent a doctor prescribing it to a patient for the unapproved use. In such a case, there is no legal requirement for ensuring that the patient is in fact, fully informed of the risks involved.

"The prescribing of an approved drug for an unapproved use by individual physicians in the practice of medicine is beyond the jurisdiction of the FDA," Dr Charles C. Edwards, Commissioner of the agency told the Kennedy subcommittee last week. He pointed out that the history of the Food and Drug Act is replete with statements that Congress does not intend the FDA to regulate the practice of medicine between the physician and his patients.

"We cannot, in good conscience, require that a physician watch a patient's condition worsen because the package insert provides for only a low dose of the drug or contains an applicable warning or contraindication," he said. And nobody who spoke at the hearings disagreed.

But what concerned Kennedy was reports that two drugs, Depo-Provera, a progesterone derivative, and diethylstilbestrol, which are approved for a variety of uses but which are supposed to be on clinical trials as birth control agents, are being prescribed in large quantities, and it seems, without sufficient safeguards to protect the rights of the recipients.

Depo-Provera has been marketed for several years for the treatment of inoperable, recurrent uterine cancer, and it has also been under clinical trials in the United States as a birth control agent since 1963. Its promise as a birth control drug lies in the fact that it needs to be administered only once every three months, by injection, and it seems to be highly effective. But in 1970 studies in dogs revealed that Depo-Provera produced mammary tumours, and according to Dr Edwards, an FDA advisory committee believes that long-term metabolic studies are still required before the drug can be approved as a contraceptive agent. Its use is, in any case, intended only for those who have tried all other birth control methods without success. In 1970 there were about 1,000 patients involved in FDA trials of the drug, but the number has now dropped to about 140.

But health officials in the state of Tennessee are not prepared to wait for the FDA to approve the drug before making it available through state-run family planning clinics. Dr Robert H. Hutcheson Jun., Assistant Commissioner in the Tennessee Department of Health, told the committee that 942 patients in Tennessee are receiving Depo-Provera from family planning programmes, and Dr James Brown, superintendent of a mental institution near Memphis later testified that the drug is administered to 181 women in his hospital to prevent pregnancy and to prevent menstruation for hygienic reasons.

Dr Hutcheson further testified that although they have the same information as the FDA, health authorities in Tennessee came to a different conclusion from the federal agency, believing that the drug is safe for use in some cases. The result is that there are now nearly ten times as many patients receiving Depo-Provera for birth control in Tennessee than are receiving it through the FDA monitored clinical trials.

A critical issue is whether the patients treated in Tennessee are informed of all the risks involved in the treatment before they give their consent. Hutcheson pointed out that they all sign a form detailing the risks, but Kennedy pointed out that the form is not as detailed as the consent forms drawn up by the FDA for their clinical trials of Depo-Provera, and Marsha Greenberger, an attorney for the Center for Law and Social Policy, provided documentation to the committee to back up her assertion that at least six patients were completely unaware of the risks.

Depo-Provera is marketed solely by the Upjohn Company, and it is thus relatively easy to control from the source. Dr W. N. Hubbard Jun., executive vice president of the company, told the Kennedy committee last week that his company has already stopped shipments to the Tennessee health department. He also said that sufficient evidence has been accumulated to make a decision on most questions surrounding the drug, and there is therefore no reason for the FDA to issue an investigatory permit to the Tennessee health department—such action would not contribute to clinical evaluation of the drug.

As for diethylstilbestrol (DES), the committee received evidence that it is being dispensed quite freely from some university health services to prevent pregnancy after intercourse. DES is approved by the FDA for several gynaecological disorders, but it has been found to be associated with cancer of the vagina in young women whose mothers had taken the drug during pregnancy. The drug has also been found to be a potent post-coital contraceptive agent, and since it is readily available in pharmacies for its approved uses, the drug has been widely prescribed to prevent pregnancy.

Dr Edwards acknowledged last week that such prescribing is taking place, and he also announced that the FDA is about to approve DES as a post-coital contraceptive in emergency cases only—after rape or incest. He added that if pregnancy does result, even after taking DES, the patient should seriously consider abortion. The announcement immediately drew protests that it would make prescribing of DES even more widespread, and that since it now carries FDA approval—albeit a very restricted approval—many women will not consider the risks involved very seriously.

Dr Edwards said in his testimony that in all cases of drug investigations on humans, the ultimate responsibility for advising patients of the risks involved, and for following up on the treatment rests with the investigator. He added that better prescribing of drugs may be brought about by continuing education of doctors, and by better peer review mechanisms. Those suggestions were generally accepted by other witnesses before the committee, and it was also generally agreed that informed consent is the central requirement in any investigational procedure.

Surprisingly, however, the case of methadone, which embodies many of the questions raised during the hearings, was not brought up in the discussions. While the drug was on trial, the FDA allowed its use to mushroom by issuing many more investigationary permits than needed just for clinical information.

Then, when the FDA approved methadone for use in the treatment of heroin addiction, it withdrew the drug from pharmacies and stipulated that it would be made available to drug clinics directly from the manufacturers, thereby depriving other doctors of the ability to prescribe an approved drug. In that case, the FDA clearly regulated the practice of medicine between the physician and his patient. Considerations of practicality aside, such actions raise a number of difficult questions.

NEWS AND VIEWS

Studying the Formation of Ocean Floors

EVERY year some 10 km^3 of molten rock are transferred from the mantle into the crust of the Earth. The eruption south of Iceland, which began on January 23 and was heralded by the opening of a fissure 1.5 km long, typifies this process. Indeed most of this igneous activity occurs along the ocean ridges from which the newly formed rocks move outwards at a few centimetres a year to form the ocean floors. Although this is undoubtedly the most important rock-forming process in the outer part of the Earth and the localities where it occurs are very precisely known, it remains a remarkably difficult phenomenon to study. The difficulty arises from the high density of the rocks involved, for these mafic and ultramafic rocks are so heavy that they are rarely uplifted to be exposed by erosion.

The problem has been attacked in three ways; by observations in the field, notably in Iceland where part of a mid-oceanic ridge lies above sea level, by geophysical studies of the deep ocean floor, supplemented by drilling and by examination of material recovered from submarine fault scarps, and by investigation of the handful of places where fragments of seafloor have been uplifted in regions where the oceanic crust has been trapped between two advancing continents. All three lines of attack produce an idealized model of an oceanic crust a few kilometres thick, having at its base mantle rocks from which the overlying igneous rocks have been derived. Above this depleted mantle follow mafic intrusions, partly gabbroic but characteristically in the form of numerous vertical dolerite dykes; at some levels these are so numerous that no other rock is present. Most of these intrusions, which collectively form an irregular layer, apparently had no connexion with the surface, but some fed an overlying succession of lavas which constitute the uppermost storey of this igneous succession. The accumulation of sedimentary rocks as this igneous sequence ages and is carried away from the ocean ridge where it originated completes a horizontally layered sequence of a kind which seems to underlie all the principal oceans.

The well-known magnetic anomalies seem to originate in the uppermost parts of this succession, implying that some process has greatly reduced the remanent magnetism of the lower parts. The recovery of metamorphosed rocks from the ocean floor and from submarine scarps has led to the suggestion that widespread metamorphism might be the cause of such changes. As Van Andel has expressed it, such a regional metamorphic layer would place a floor under the zone responsible for the observed geomagnetic anomaly patterns.

The Troodos mountains in Cyprus were first identified as a possible upthrust slice of oceanic crust and mantle by Gass and Masson-Smith in 1963. On page 26 of this issue of *Nature* Gass and Smewing further develop this hypothesis and in particular describe the work they have carried out from the Open University on the zeolites and their distribution throughout the Troodos massif. The new evidence provides further indications of how the Cyprus rocks can, in conjunction with the volcanic successions developed in Iceland, be employed to give what is probably the most complete model deduced from exposed rock or an ocean ridge in cross-section. With the aid of such a model one can tackle two problems: How did the rocks form in the first place? What modifications are likely to have occurred subsequently? A critical part is played by the vertical dykes which at depth make up the whole Troodos complex in Cyprus, and in Iceland, where higher sections are exposed, increase in number downwards through the volcanic succession. The discovery of this remarkable structure, essentially a pile of volcanic rock underlain by ever increasing numbers of dolerite dykes, was made almost simultaneously by Wilson in Cyprus and by Walker in Iceland. Both appreciated the significance of what they had found, and realized that the presence of vertical dykes indicated a considerable extension of the crust. Walker postulated a 400 km extension below Iceland, and Wilson commented "the sialic crust appears to have moved away under great tensional stress while the numerous dykes were extruded". Such interpretations, made in the late 1950s before Hess's statement on seafloor spreading, were remarkably prescient comments.

Gass and Smewing record the metamorphic changes they have observed in Troodos. The zeolite zones they recognize correspond to the deeper zones identified in Iceland. In Cyprus these pass downwards into green schist rocks metamorphosed at still higher temperatures. The nature of the igneous rocks of the Troodos Complex has already suggested that a deeper section through old oceanic crust is exposed than is found in the Tertiary rocks of Iceland. The new evidence on the metamorphism in Cyprus confirms this relationship. Among the interesting matters that Gass and Smewing have established is the fact that an early series of volcanic rocks had been metamorphosed and then eroded in part before a later series of pillow lavas accumulated.

A general point that Gass and Smewing develop is the possibility that physical changes produced by metamorphism may account at least in part for some of the layered structure which characterizes ocean floors. In this they are following up earlier work in the Atlantic and on Iceland, where, for example, Gibson and Piper showed that increases in density produced by the development of zeolites in volcanic rocks could account for the properties of layers 1 and 2 indicated by seismic studies below Iceland.

Crust is formed at a spreading ridge in two different though closely related fashions. The vertical dolerite dykes, arranged like a pack of cards on edge, increase in number as movement continues and so build up a

layer, possibly layer 3 of the ocean floor. The overlying layers 1 and 2 seem to be constructed partly of dykes and partly of lava flows, the proportion changing as one goes upwards through the crust. In Iceland, where such lavas have been most closely studied, they are stacked like shingles on a roof dipping inwards at 5 to 10° towards the centre of the island. It is not at all easy to attach individual flows to the dykes that supplied them. Fortunately, very distinctive features are present, which enable one to learn something of the way in which the volcanic rocks develop. At local centres within the Icelandic lavas an unusual variety of magmas are erupted; the resulting lavas include acid rocks, readily identified among the predominantly basaltic pile; their distinctive nature and the way in which they increase in thickness toward the centre from which they were erupted make it possible to identify several central volcanoes.

The work of Icelandic and British geologists has shown how these centres remain active for about a million years during which time they must be transported many kilometres outwards from the spreading axis. In most instances they then become extinct and their place is taken by a new volcanic centre formed near the ridge, which in turn is displaced for a few tens of kilometres, erupting volcanic rocks as it goes. In this way, as Gibson and Piper (*Phil. Trans. Roy. Soc. Lond.*, **A271**; 1972) have shown, successive groups of lavas, each with its attendant central volcano, come into being and are transported outwards and overlapped by younger lens-like units of lava flows. J. S.

Bacterial Polarity

THE central feature in theories of how genes are controlled, both in bacteria and in the cells of higher organisms, almost always consists of a model to explain how their transcription into messenger RNA is induced or repressed. But in spite of the considerable progress which has been made in tracing formal control networks and defining their biochemistry, details of the enzymatic processes by which RNA chains are initiated, elongated and terminated have remained elusive. De Crombrugghe and his colleagues at the National Institutes of Health, in their earlier articles, concentrated their attention upon the control of bacterial operons at the initiation of transcription (see *Nature New Biology*, **231**, 139; 1971). They now report, in last Wednesday's issue of *Nature New Biology*, **241**, 260; 1973), that they have extended their studies to the termination of transcription, which may provide a hitherto unsuspected control of gene expression in bacteria.

Initiation of transcription usually seems to be the crucial step in controlling gene activity. Initiation in general depends on one polypeptide component of the bacterial RNA polymerase, the sigma factor. In many bacterial operons, however, an additional control protein—which is activated by cyclic AMP—must be present to assist the initiation of RNA synthesis. This interaction, coupled with the inhibition of transcription by repressor proteins, accounts for the control of expression of inducible and repressible operons. Uninfected bacterial cells contain only one sigma factor, but when phage DNAs infect a host cell they may cause the replacement of the sigma factor of the host polymerase by a phage-coded protein which changes the specificity of transcription.

Termination of transcription has more recently begun to seem a plausible mechanism for controlling gene expression during phage infection; it now seems almost certain that one of the first products specified by an infecting phage DNA is an anti-terminator protein which allows the host polymerase to "read through" sites at which it would otherwise terminate on the phage genome. The bewildering multitude of sigma factors seems to have been an artefact, for the introduction of changes in the initiation machinery of the host takes place only at a later stage.

Termination is catalysed by one of the innumerable protein factors of macromolecular metabolism in bacteria, the rho factor. Since the discovery in bacterial cells of this component which acts as a template of phage DNA (and which is antagonized by the anti-terminator proteins synthesized during infection), the involvement of this protein in RNA synthesis in the host bacterium has been a considerable puzzle. De Crombrugghe *et al.* now provide the first demonstration that rho factor is active with bacterial DNA.

The three genes of the galactose system of *Escherichia coli* are transcribed in the order *galE-T-K* both *in vivo* and *in vitro* when they are part of a phage lambda DNA molecule. When RNA polymerase is allowed to transcribe this template in the absence of rho factor, very large molecules of RNA are synthesized, presumably because the enzyme reads past the end of the galactose operon into the regions of phage DNA. When a low concentration of rho is added, however, the largest RNA molecules sediment at about 22–25S, which corresponds to the size of the transcript expected from the complete *gal* gene cluster; the smallest RNA molecule in this gradient sediments at about 12–15S, which is about the size expected of a transcript of the first gene, *galE*, alone. Hybridization experiments in which the RNA product is annealed to denatured DNAs derived from phage genomes which carry only part of the galactose operon confirm the idea that the large molecules of RNA correspond to *galETK* and the small ones to *galE* alone. This suggests that low concentrations of rho cause some polymerase molecules to terminate transcription at the end of the *galE* gene, although others continue past this site to a second termination sequence located at the end of the operon. When the concentration of rho is increased, all the polymerases terminate at the first site, for the sole product of the reaction is an RNA of about 14S corresponding to *galE* alone.

The traditional view of transcription of bacterial operons is, of course, that they are synthesized into long polycistronic mRNAs—which represent the sequences of all the genes of the operon. These messengers are sequentially translated into the various proteins for which they code. Termination of transcription within an operon is not succeeded by reinitiation and synthesis of a messenger for the remaining genes, for De Crombrugghe *et al.* find no transcripts corresponding to *galTK*. If conditions *in vitro* reflect those which prevail *in vivo*, internal termination may cause the production of more messenger sequences for early genes in the operon, leading to increased synthesis of their protein products relative to the later genes. On the other hand, the presence

of internal termination sites may reflect the evolution of gene clusters from independent genes and they may be ignored by the rho factor in the conditions prevailing *in vivo*.

An important difference between the conditions of transcription *in vitro* and *in vivo* is that translation proceeds simultaneously with transcription in the cell, but the messengers are not translated *in vitro*. One consequence of the failure of simultaneous transcription and translation in the cell is polarity ; mutants which cause termination of protein synthesis in one gene change the properties of messenger RNA representing subsequent genes. Two theories have been proposed to explain this polarity. One explanation is that messenger is synthesized beyond the mutant site but is degraded by cellular nucleases because it is no longer being translated ; the other postulates that translation is needed if transcription is to continue so that the enzyme ceases RNA synthesis at or soon beyond the mutant site (see *Nature New Biology*, **232**, 161 ; 1971).

Polarity can be caused by the presence of nonsense mutations and by the insertion of foreign DNA into the operon. Insertion mutants differ from nonsense mutants in that they are always extremely polar and their polarity does not show the dependence on position within the gene characteristic of nonsense mutations. De Crombrugghe *et al.* have found that one such insertion mutant where the insertion is located close to the beginning of the galactose genes is transcribed normally *in vitro* by RNA polymerase. But if rho factor is added to the incubation, transcription terminates within the inserted sequence. The insertion is very sensitive to rho and reacts at even the lowest concentrations of the factor.

In this situation, polarity results from rho-dependent termination. Does a similar mechanism explain the polarity of nonsense mutations, perhaps, for example, because nonsense codons constitute part of the DNA sequence recognized by rho factor? Polar nonsense mutants in the galactose operon proved to have no effect on transcription, however, in either the absence or presence of rho. This implies that the polarity of nonsense mutants depends on the failure of translation as such and not on the sequence of the nonsense codons themselves.

How widespread is the use of rho in bacterial operons? At least one other operon, the lactose operon, contains rho-sensitive signals, for De Crombrugghe *et al.* find that low concentrations of rho seem to cause transcription to halt at the end of the operon ; but high concentrations generate a small RNA product, sedimenting at about 12–14S, which is of the size expected to correspond to only the first one-third or so of the *z* gene, the first gene of the operon. The correspondence of this location with a peak in the gradient of polarity (a region in which polar mutants have less effect upon the expression of subsequent genes) suggests that termination does not result from rho action at a site which by chance resembles true terminator sequences ; it seems likely that the action of rho *in vitro* reflects the organization of the *z* gene *in vivo*. One possible implication is that the *z* gene may in fact comprise two genes, not one as has previously been thought.

The immediate significance of rho-dependent termination within an operon is that this mechanism may explain the natural polarity of some operons, in which later genes direct synthesis of less protein than earlier genes. This might be achieved by utilizing signals at the ends of genes which have lower affinities for rho than those at the ends of genes; rho recognition signals may yet prove to be present at the ends of all genes in an operon. Another variation on this theme is to suppose that there might be different kinds of rho factor to recognize the different signals. Conditions in the cell may differ appreciably from those *in vivo* so that defining the function of the rho factor as a cellular control protein must demand the isolation of mutants in the termination protein. De Crombrugghe *et al.* say that their next experiments will be to test the intriguing speculation that rho-dependent termination may provide an alternative explanation for the polarity of nonsense mutants ; perhaps internal rho-dependent termination sites are activated when translation ceases at a previous nonsense mutation. B. L.

Cell Cycle in *Xenopus*

BETWEEN the stages of early gastrula and late neurula profound changes take place in the *Xenopus* embryo ; the cells undergo about three divisions, increasing seven to eight times in number, and differentiated tissues such as notochord, neural tube, muscle somites and gut become recognizable histologically. But what would happen, one may ask, if mitosis is inhibited at the early gastrula stage, so that there can be no increase in cell number? The results of this intriguing experiment are reported on page 55 of this issue of *Nature* by Jonathan Cooke, of the University of Sussex.

Rather surprisingly Cooke found that nothing very drastic does happen to the development of the embryo if cell division is totally and rapidly inhibited at the early gastrula stage, either with colcemid or mitomycin C. The late neurula has all the differentiated tissues and morphology of the normal embryo, but only about one eighth the number of cells, which are correspondingly larger.

By itself this result is interesting in that it suggests that cells in the embryo do not have to go through a fixed number of divisions or normal chromosomal replications before differentiating. It does not, however, exclude the possibility that cells have to traverse part of the normal cell cycle to be able to respond to a change in positional information, a suggestion which has come from work on the insect cuticle. To test this hypothesis, Cooke transplanted a dorsal lip organizer into an inhibited embryo. This operation resulted in a second site of ectodermal invagination and the development of a second neural tube and notochord. In this way host cells were committed to a completely different developmental fate even though they could not undergo cell division. Experiments are in progress to test whether DNA synthesis and abnormal chromosome replication are still taking place in the colcemid-inhibited cells, and whether these processes are absolutely required for morphogenesis and differentiation.

From a Correspondent

VISION

Visual Feedback

from our Animal Behaviour Correspondent

AN ingenious series of experiments carried out on kittens during the past 10 years at the Massachusetts Institute of Technology establishing the conditions necessary for the development of normal visual-motor coordination have shown that kittens require visual feedback from the result of their own movements. Kittens moved passively through the environment without experiencing the visual consequences of moving their limbs remain ill coordinated, unable to direct their movements and apparently with little idea of the spatial configurations of the environment.

In the latest of this series of studies, A. Hein and R. M. Diamond (*J. Comp. Physiol. Psychol.*, **81**, 394; 1972) have attempted to separate two components of this visual feedback: "seeing arm limb movements" and "seeing environment change as result of own movement". Their results suggest that it is not sufficient for a kitten to see its limbs move in isolation from the rest of the environment; only if the kitten has also experienced some other aspects of its visual environment in relation to its own movements will it be able to perform such tasks as reaching out a paw and touching a bar.

Hein and Diamond gave kittens experience of visual feedback from their paws in isolation from other sorts of visual experience by putting a spot of luminous paint on one paw and keeping the kittens in the dark. In this way, the only thing they could see was a patch of light as their own paw moved. Perhaps not surprisingly, after 10 days of this, the kittens gave evidence of being very ill coordinated, unable to reach out towards objects, and bumping into obstacles. They were then allowed experience of a normally illuminated room. Half of them had opaque collars on so that, although they could move freely about, they could not see their own limbs and so did not receive visual feedback from their own limb movements. The other half were not only prevented from seeing their own limbs but were restrained in a holder, so that they could only see what was going on around them. After 10 days of this treatment, none of the kittens showed visually guided reaching—that is, when they were lowered towards a ladder, they did not reach out their paws towards the rungs. But the kittens that had been allowed to move freely were superior in one respect: they avoided obstacles when moving around, unlike the restrained kittens. All kittens were then given a further 10 days of the luminous paw treatment, but this time the spot of paint was put onto the opposite paw from that in their initial exposure. As before, the only thing the kittens could see during this time was the spot of paint on their paw, as the rest of their environment was in total darkness.

It was after this treatment that the difference in reaching ability between restrained and unrestrained kittens became apparent. Only the animals that had previously developed visually guided locomotion (the ability to avoid obstacles by visual means, for example) showed properly developed visually guided reaching. What was even more remarkable was that they could reach correctly only with the paw which had had the spot of paint on it and from which they had had visual feedback, in the third part of the experiment. They were unable to guide their other paw correctly. Kittens which had been restrained and had never developed the ability to move around without bumping into things could not reach correctly with either of their paws.

Previous studies had shown that in the kitten, visual feedback from a moving limb is essential for the development of properly coordinated visually guided reaching. This experiment has added a proviso: it would seem that visual feedback from the forelimb is sufficient for the acquisition of visually guided reaching only if visually guided locomotion around the environment has also been developed.

PROTEINS

Hunting the Hybrid

from our Molecular Biology Correspondent

ONE of the unswept corners of haemoglobin chemistry, which has irked the practitioners for some time, is the balance of species present in a mixture of two haemoglobins, such as occurs in sickle-cell trait blood. Electrophoretically, normal adult and sickle-cell haemoglobins, $\alpha_2^A\beta_2^A$ and $\alpha_2^A\beta_2^S$, separate easily enough, but because the haemoglobin tetramer is in rapid equilibrium with its symmetrical $\alpha\beta$ dimer, there should be every reason to expect the hybrid $\alpha_2^A\beta^A\beta^S$ to be represented in the mixture. This, however, is an elusive creature, which escapes its pursuers by dissociating during the fractionation process: the hybrid will have an electrophoretic mobility between those of the parent species, haemoglobins A and S. The dimers $\alpha^A\beta^A$ and $\alpha^A\beta^S$, in equilibrium with the hybrid tetramer (no matter in how low proportion), will accordingly separate from the latter, one ahead and one behind, and turn respectively into $\alpha_2^A\beta_2^A$ and $\alpha_2^A\beta_2^S$ tetramers. Unless then the dissociation to dimers can be inhibited, the existence of the hybrid, like the extinguished light in the refrigerator when the door is shut, can only be taken on faith. Macleod and Hill (*J. Biol. Chem.*, **248**, 100; 1973) have now finally found a method, using a covalent cross-linking reagent, to salt the beast's tail.

Oncogenic Potential of *Herpesvirus saimiri*

Herpesvirus saimiri, which is indigenous to squirrel monkeys and which has not been associated with any disease, malignant or otherwise, in animals of this species, has of late attracted the attention of tumour virologists because when it is inoculated into other primates—marmosets, ringtail, owl and African green monkeys, for example—it induces lymphomas or leukaemias. Virtually nothing is known to date about the molecular biology of this oncogenic herpesvirus, but as Ablashi and seven colleagues report in *Nature New Biology* next Wednesday (March 7), the oncogenic potential of this virus remains after the virus has been heat inactivated such that it can no longer replicate and kill owl, monkey and African green monkey cells.

Ablashi *et al.* propagated and isolated infectious *Herpesvirus saimiri* in Vero cells, a stable line of cells derived from African green monkey tissue. They then inactivated the viral particles by heating them to 56° C for 30 min. Intact viral particles were not morphologically changed by this treatment although the capsids of unenveloped particles appeared to collapse during heating. After this treatment the virus failed to produce any cytopathic effect in Vero cells or in owl monkey cells. Four owl monkeys, however, after receiving six weekly injections of the inactivated virus, eventually developed malignant lymphomas the pathology of which is described in some detail by Ablashi and his colleagues. Furthermore, infectious *Herpesvirus saimiri* could be recovered by co-cultivating, with Vero cells or owl monkey cells, tissues collected from the lymphomatous owl monkeys at necropsy.

It seems, therefore, that *Herpesvirus saimiri* inactivated by heat so that it is no longer able to kill susceptible host cells may retain not only the ability to transform cells and induce tumours but also retain the genetic information required to code for complete infectious progeny viral particles. The precise nature of the molecular mechanisms that underlie these phenomena remains unknown, but it seems clear that the cytocidal and oncogenic potential of this virus can be separated.

Internal cross-links were introduced in the tetramers of a haemoglobin A and S mixture with a bifunctional fluorodinitrobenzene, specifically *p,p'*-difluoro-*m,m'*-dinitrodiphenylsulphone. Gel electrophoresis after reaction revealed a third component, running between the A and S zones. This is not, of course, proof of the presence of a hybrid, for the annihilation of amino groups—actually as shown in earlier work, the α-chain termini — by the cross-linking reagent renders any identification based solely on electrophoretic mobility uncertain. Macleod and Hill therefore went on to isolate the hybrid in the following way: concentrated magnesium chloride was added to the mixture after reaction, so as to dissociate all dissociable haemoglobins into $\alpha\beta$ dimers. Any molecules cross-linked across their α or their β chains are unable to dissociate, and this fraction, which seems to be rather more than half of the total in the conditions used, is collected by gel filtration. Ion-exchange chromatography of this material gives rise to three principal fractions. By contrast the undissociable fraction in haemoglobins A and S mixed after separate exposure to the cross-linking agent generates only two components, corresponding to the first and the third species eluting from the heterologous system. The inference is that the middle peak is the cross-linked hybrid, $\alpha_2^A\beta^A\beta^S$, and it is shown to be such by analysis of the product of trypsin hydrolysis, in which the characteristic β^A and β^S peptides are present in equal amount.

The concentration of the cross-linked hybrid formed in an equimolar mixture of haemoglobins A and S is actually considerably greater than that of either of the homologous hybrids, and it therefore seems that the hybrid tetramer is not merely a significant, but in fact the preponderant, component in sickle-cell trait haemoglobin at equilibrium. This gives substance to a suggestion concerning the low probability of sickling in erythrocytes of sickle-cell heterozygotes, compared with homozygotes. It has been conjectured that the polymerization of the haemoglobin S in the cell is impeded by the incorporation of hybrid tetramers into the aggregates.

A different use for bifunctional reagents is as a means of restricting structural adjustments between the haemoglobin subunits during the oxygenation process. Cross-links within the β chains have been found sufficient to inhibit the transition to the low-affinity state. An interesting new essay in this direction comes from Fasold, Meyer and Steinkopff (*Europ. J. Biochem.*, **32**, 63 ; 1973), who have used a reagent based on iodoacetamide, with the built-in advantage that it can be cleaved in the middle by reduction of an azo link. The reagent is *p*-bis-iodoacetamide-2,2'-dicarboxyazobenzene; it is rigid, and can therefore only join reactive groups separated by a prescribed distance with rather little tolerance. Rapid reaction occurs with haemoglobin, with uptake of two moles of reagent per tetramer, after which a slower reaction supervenes.

The product of the first reaction, when fractionated on an ion-exchange column, contains one major component, which is tetramic, and possesses two α and two β chains, with one cross-link per β chain, for in a hydrolysate two groups in either are found to be carboxymethylated. The cross-link, which joins cys-93 to lys-82, is submerged in the molecule, more or less under the F helix. A minor product of the reaction also contains two cross-links, which, however, are asymmetrically disposed, one lying within a β chain, as in the major product, the other linking the cys-93 of the second β chain to his-45 of the neighbouring α chain. The corresponding monofunctional reagent blocked only the cys-93 of each β chain. The introduction of this foreign body into the vitals of the haemoglobin molecule caused a diminution in the haem-haem interaction, with a drop in the Hill constant to 1.7. With cross-links in the β chains, on the other hand, the Hill constant dropped to unity, and the same was found for the asymmetrically reacted species, in which only one β chain contains a cross-link. The authors suggest that the transition between the high and low-affinity conformational states may be repressed with some degree of independence at

DNA Synthesis in Developing Sea Urchins

IN next Wednesday's *Nature New Biology* (March 7), Infante and his colleagues report the isolation from the nuclei of developing sea urchin embryos of a DNA-membrane complex which supports DNA synthesis both *in vivo* and *in vitro*. The natural synchrony of the first few cellular divisions after fertilization of the sea urchin egg facilitates study of the different stages of the division cycle.

Nuclei were isolated from embryos containing completely labelled DNA. The nuclei were lysed with detergent and the lysate was centrifuged on a discontinuous 15–40 per cent sucrose gradient. The bulk of the DNA was found free at the top of the gradient, but 10–30 per cent formed a band (the M-band) at the 40 per cent sucrose layer and was associated with membrane material. Treatment of the M-band complex with DNase, RNase, pronase, phospholipase-C and deoxycholate indicated that DNA, RNA, protein and phospholipids were all important in maintaining the integrity of the M-band.

When embryos in S-phase were pulse labelled with ^{3}H-thymidine for 30 s at 17° C, more than 70 per cent of the labelled DNA was located in the M-band. The remaining labelled DNA, located in the top fraction of the gradient, was demonstrated to be released DNA originating in the M-band when the labelling experiment was repeated at 5° C, at which temperature DNA synthesis is greatly retarded. At 5° C, all the DNA synthesized in a 30 s pulse was localized in the M-band. Although, following an extended labelling period of 10 min, the bulk of the DNA was found in the top fraction of the gradient, all of the DNA made in a 330 s pulse at the end of this 10 min period was located in the M-band. Thus DNA replication seems to occur at the membrane and non-replicating DNA is located in a fraction which is more readily dissociated from the membrane complex.

Further experiments, in which embryos were labelled for 35 min starting in the S-phase and ending in G2, showed that, as the cells entered G2, virtually no DNA could be found associated with the M-band. Thus the formation of the DNA-membrane complex may be a prerequisite for DNA synthesis. Furthermore, no M-band fraction could be found in mature unfertilized eggs—cells in which there is no DNA synthesis. When unfertilized eggs were mixed with embryos which had received a 30 s pulse during S-phase, an M-band fraction was obtained, showing that the absence of M-bands in unfertilized eggs was real. Thus the M-band seems to be formed soon after fertilization when DNA synthesis is initiated and disappears in G2 when synthesis is complete.

The M-bands and top fractions were isolated from early blastula nuclei and tested for their capacity to support DNA synthesis *in vitro*. No activity could be detected in the top fractions but the M-band fraction supported the synthesis of DNA which was dependent on the presence of all four deoxyribonucleotides.

These experiments must be interpreted with care. As suggested recently by Huberman *et al.* (*Nature*, **241**, 32 ; 1973) and Fakan *et al.* (*Proc. US Nat. Acad. Sci.*, **69**, 2300 ; 1972) during cell fractionation experiments it is possible that newly replicated DNA may possess special features such as single strandedness which may cause it to bind more protein or membrane material than bulk DNA. In other words, the M-band may be an artefact of cell lysis.

the level of tertiary or of quaternary structure, that is to say by intra- or inter-chain cross-links.

LEAF MICROFLORA

Disease Control

from a Correspondent

FACTORS which influence the ecology of microorganisms on leaf surfaces were considered at a meeting of the Pesticides Group of the Society of Chemical Industry held on January 15 under the chairmanship of Dr E. Evans (Chesterford Park Research Station). The interrelationship of these factors in relation to practical disease control was indicated by several of the speakers.

The nature of the leaf surface and its microclimate may determine the success or failure of organisms to colonize foliage and these factors were surveyed by Dr B. E. Juniper (University of Oxford) and Dr S. W. Burrage (Wye College, University of London), respectively. Dr Juniper's electron micrographs of the leaf surfaces showed the marked variation in thickness and nature of cuticles and epicuticular waxes, not only between species but also between abaxial and adaxial surfaces of individual leaves. The wettability of leaf surfaces is influenced by the fine structure and chemical composition of the wax. Dr Burrage stated that the temperature and humidity at the leaf surface are determined by the balance between heat transfer by radiation, conduction and evaporation/condensation of water. The shape, size, orientation and surface topography of the individual leaf influence the boundary layer thickness and hence the energy exchange and microclimate.

The effect of the latter on spore germination and the growth of leaf microorganisms was also stressed by Dr J. P. Blakeman (University of Aberdeen). Water on foliage can release substances from leaves, especially older ones that have lost wax by weathering, and from microorganisms. He mentioned that pollen deposits on leaves stimulate spore germination and growth of *Botrytis cinerea* and of the saprophytic bacterium *Sporobolomyces pararoseus*. The latter reduced development of *Phoma betae* lesions on sugar beet foliage, probably by competing for nutrients.

Dr P. J. W. Saunders (University of Manchester) discussed the effects which pollutants may have on leaf surface microflora; for example, *Dilocarpon rosae*, which is sensitive to sulphur dioxide, will be favoured by a reduction of this pollutant as indicated by the increasing incidence of rose black spot in some areas where clean air zones have been enforced. Dr C. H. Dickinson (University of Newcastle upon Tyne) stated that bacterial populations on foliage usually increase as leaves age. He summarized the effects of various fungicides on microflora on the surfaces of potato and barley leaves. Disturbance of the ecological system by the interactions of fungicide with different microorganisms might produce beneficial or adverse effects on the plant. One fungicide, which decreases populations of saprophytic organisms on the leaf surface, was associated with delayed foliar senescence and increased barley yields even in the absence of recognized pathogens. Dr R. T. Burchill reviewed work at East Malling Research Station showing that apple scab, caused by *Venturia inaequalis*, can be controlled by spraying orchards with a 5 per cent solution of urea after harvest but before leaf fall. He stated that the mode of action of urea is complex: it may act partly by increasing leaf populations of fungi such as *Alternaria* spp., *Cladosporium* spp. and *Fusarium* spp., all of which antagonize the development of *V. inaequalis* perithecia; moreover, urea stimulates some soil flora and increases the rate of decomposition of fallen leaves, thus decreasing the carry-over to the next season of infection by perithecia.

Dr G. Barnes (Chesterford Park Research Station) has investigated the interaction of *Erysiphe polygoni* and *B. cinerea* on detached leaves of clover, *Trifolium pratense*. Dry conidia of *B. cinerea*, when placed on the leaf surface 24 hours before inoculating with *E. polygoni* conidia, slightly decreased germination and formation of the appressorium in the latter and reduced the production of secondary hyphae by as much as 80 per cent. No such effects occurred when conidia of *E. polygoni* were inoculated either before or simultaneously with *B. cinerea* conidia. Interactions between bacterial populations on leaf surfaces were surveyed by Dr J. E. Crosse (East Malling Research Station). He has found that inoculation of apple shoots with *Erwinia amylovora*, the bacterium causing fireblight, immediately after inoculation with a yellow bacterium, which is commonly associated with fireblight lesions, decreased infection by *E. amylovora*. Similarly, the rate of infection and spread of cherry canker, caused by *Pseudomonas morsprunorum*, is diminished by a white bacterium isolated from cherry leaf surfaces. Attempts to use saprophytic bacteria as a satisfactory biological control against these diseases have, so far, failed because their populations rapidly fall to levels non-competitive with the pathogens.

SUPERFLUIDS

Building Up the Case

from a Correspondent

IT now seems much more probable that there is a third superfluid in nature, in addition to liquid ^{4}He and the electron gas in superconductors. This is the implication of a recent report by Webb, Greytak, Johnson and Wheatley (*Phys. Rev. Lett.*, **30**, 210; 1973), the latest in a series of papers from both

DNA Polymerase Mutant of the Smut Fungus

SINCE the isolation by DeLucia and Cairns of mutant strains of *Escherichia coli* lacking DNA polymerase I activity two other DNA polymerases of *E. coli* have been identified and characterization of the biochemistry of DNA replication continues apace. Of course, it should be possible to make essentially similar analyses of the DNA replication machinery of at least some eukaryotes, and with this aim in mind Jeggo *et al.* screened several hundred temperature sensitive mutants of the smut fungus *Ustilago maydis*. As they report in *Nature New Biology* next Wednesday (March 7), they found five temperature sensitive mutants of *U. maydis* that were blocked in DNA synthesis at the non-permissive temperature.

DNA polymerase activity in extracts of one of these mutants was only 10–25 per cent that of wild type. Tetrad analyses indicated that the temperature sensitive phenotype of this mutant is the result of a single recessive mutation which may well be in a structural gene for a DNA polymerase because partially purified enzyme from the mutant proved to be more thermolabile than wild type enzyme. Susceptibility to radiation damage and to chemical mutagens of the mutant strain of *U. maydis* growing at different temperatures suggests that the mutation does not affect a principal DNA repair pathway. Because, however, the mutant cells, grown at the non-permissive temperature, develop into filamentous uninucleate forms whereas wild type cells grow by budding like a yeast, it may well be that Jeggo *et al.* have been lucky and have isolated a temperature sensitive mutation which affects DNA polymerase involved in chromosome replication.

Confirmation of this possibility will depend on the results of experiments which may lead to a characterization of the replication machinery in this fungus.

sides of the Atlantic which suggest with increasing certainty that a phase transition occurs in liquid ^{3}He near 0.002 K. This transition is closely analogous to the superconducting transition in a metal.

According to quantum statistical mechanics, the properties of a fluid at very low temperatures are determined chiefly by whether the atoms of which it is composed contain an even number of fundamental particles, in which case they are bosons, or an odd number, in which case they are fermions. The ^{4}He atom (2 protons, 2 neutrons, 2 electrons) is thus an example of a boson and the ^{3}He atom, with one less neutron in the nucleus, is a fermion. The two liquids are therefore expected to display completely different properties at very low temperatures, a prediction which was verified experimentally several years ago when ^{3}He first became available in quantities sufficient for experiments. Superfluidity in liquid ^{4}He is associated with a curious phenomenon, known as Bose–Einstein condensation, in which a substantial proportion of the atoms congregate in the same zero-energy quantum state. By contrast, the occupation of a single quantum state by more than one fermion is rigorously forbidden, so that a Bose–Einstein condensation, leading to superfluidity, cannot occur in liquid ^{3}He.

There is, however, a completely different mechanism by which the electrons (also fermions) in a superconductor are able to acquire their superfluid properties. This is the formation of so-called Cooper pairs: where there is an attractive force between the electrons, on account of their interaction with the lattice of positive ions, the assembly can reduce its total energy by forming pairs of electrons. The scattering of electrons by lattice defects or thermal vibrations, which gives rise to electrical resistance in a normal metal, would entail breaking pairs and thus cannot occur unless a certain minimum amount of energy is available. The electrons can therefore flow through the lattice without dissipation of energy, provided that a critical drift velocity is not exceeded. In the case of liquid ^{3}He, the possibility of attractive interactions, which might enable a similar transition to the paired state to occur, was considered more than a decade ago by several workers who estimated transition temperatures around 0.1 K. Subsequent experiments failed to reveal a transition; but more refined theories quickly pushed the predicted transition to still lower temperatures. This cyclical process has been repeated several times since then, but on each occasion the transition has failed to appear at the temperature expected.

The whole topic attracted renewed interest last year when Osheroff, Gully, Richardson and Lee (*Phys. Rev. Lett.*, **29**, 920; 1972) reported evidence of some sort of phase transition in liquid ^{3}He under high pressure near 0.002 K. Unfortunately, however, their data were hard to interpret in detail because their cooling technique meant that, inevitably, some solid ^{3}He was also present in the experimental cell.

The experiments by Webb *et al.* made use of a different cooling method, and provide the first clear indication that the phenomenon observed by Osheroff *et al.* is indeed the long-awaited transition to a paired state: they find that no latent heat is associated with the transition, but that there is a finite discontinuity in the specific heat. This behaviour is characteristic of a second-order phase transition, the only other known example of which is the superconducting transition in a metal.

Does the new phase of liquid ^{3}He have superfluid properties? Observation of the second-order phase transition leaves little doubt that there are close similarities between this new phase and the electrons in a superconductor, but clearly there are also profound differences. In a superconductor the fermions are negatively

Integrating Two Unrelated DNA Sequences

MOST bacteriophage DNAs which can be inserted into the chromosome of a bacterial host on infection can integrate at only one, or at least a very few, sites in the bacterial genome. A striking exception is provided by phage mu, which can integrate at any point in the chromosome of *Escherichia coli*. When it inserts within a gene, the bacterium behaves as mutant lacking the gene function. This unusual activity has already been put to practical use by Nomura and Edbaek (*Proc. US Nat. Acad. Sci.*, **69**, 1526; 1972) to map the relationship of the genes coding for ribosomal proteins and by Louarn, Bird and Caro (*J. Mol. Biol.*, **70**, 549; 1972) to demonstrate the bidirectional replication of the *E. coli* chromosome.

The mechanism of this integration is analysed in an article by Toussaint and Faelen in *Nature New Biology* next Wednesday (March 7). They have taken advantage of their previous observation that phage mu can promote the integration in the bacterial chromosome of a defective lambda phage (which usually inserts at one specific site) unable to integrate of its own accord. If the lambda phage carries an active *gal*$^+$ gene, its integration is marked by the restoration to infected cells (of the *gal*$^-$ genotype) of ability to metabolize galactose as a carbon source.

The question which Toussaint and Faelen answer is whether mu promotes the integration of lambda simply by providing some enzymatic activity or whether the two DNAs are physically linked in some way so that lambda is inserted at the same site as mu. They have therefore isolated cells in which λ*gal*$^+$ has been inserted within a bacterial gene (by screening for the inability of the cell to provide the metabolic activity coded by that gene). The next step in the analysis is to see which of the integrated phages is lost if the bacterial gene bearing these insertions is replaced by a normal, functional bacterial gene. This experiment is performed by transducing the cells with a P1 phage.

In a *his*$^-$ *E. coli* strain which had received λ*gal*$^+$ and mu, transduction to provide a normal *his*$^+$ bacterial gene resulted in the loss of both λ*gal* and mu. This implies that both phage DNAs must have integrated at the same site, within the *his* gene. In further experiments, the chromosomes of cells bearing λ*gal*$^+$ were transferred by conjugation into *gal*$^-$ bacteria. All the *gal*$^+$ recipients received both the lambda and mu DNAs, which supports the idea that mu enables lambda to integrate because the two phages are linked together in some way. In these different strains, the *gal*$^+$ was located at widely differing points in the donor chromosome, showing that mu retained its usual capacity to integrate at any point, the integration of λ*gal* taking place at the same site.

The model which Toussaint and Faelen propose to explain these results is to suppose that two phage mu DNAs are implicated in the integration process. The two mu DNAs interact with each other at the specific sites which the phage uses for integration to form a dimer. One of the two sites of this dimer integrates in *gal* DNA; the other integrates in the bacterial chromosome. The result is that the bacterial insertion site carries a λ*gal* genome, surrounded on each side by a mu genome. Studies of the structure of the DNA which is inserted support this model. The use of mutants in phage mu shows that the same phage functions are implicated in its interaction with λ*gal* and with bacterial DNA.

One implication of this model is that in principle it should be possible to utilize mu to connect together any two circular pieces of duplex DNA in the bacterial cell. Although this model is consistent with the genetic data, biochemical confirmation of the proposed DNA interactions will be needed before it can be put to such interesting and important uses.

charged electrons moving through a lattice of positive ions whereas, in the case of ^{3}He, there is no lattice and the fermions are neutral atoms. It is not obvious, therefore, just how far the analogy can be taken.

SOLID STATE

Laser Writing

from a Correspondent

THE past four or five years have seen a great increase in research on amorphous semiconductors. This has followed a rather longer period devoted to crystalline materials and the development of very sophisticated semiconductor devices. Amorphous materials have been investigated principally in the form of glasses, and the particular property that has, perhaps, stimulated the most interest is their electrical behaviour. Devices made from such materials show either memory or threshold switching depending on their composition. (Memory switching is the phenomenon by which the material may exhibit either a high or a low resistance state.)

It is now fairly certain that memory switching is associated with the transition from amorphicity to a crystalline state, in the form of thin filaments between the electrodes caused by electrical heating followed by slow cooling. The reverse process involves heating followed by a rapid cooling or quenching to allow the material to devitrify or revert to the amorphous state.

It is now possible to induce a similar condition in amorphous materials by the interaction with light from lasers. Rapid crystallization and equally rapid devitrification of amorphous chalcogenides (for example $Te_{81}Ge_{15}Sb_2S_2$) have been observed when they are exposed to short laser pulses (*Appl. Phys. Lett.*, **18**, 254 ; 1971). This process of optical switching is made evident by the sharp change in the optical transmission and reflexion properties of those areas so exposed. It was suggested that the physical change is attributable to a bond-weakening mechanism or photocrystallization process, but a more recent paper (*Appl. Phys. Lett.*, **22**, 48 ; 1973) gives evidence that in a similar glass the initial devitrification is temperature activated. This paper also shows how by using the same laser it is possible to produce a crystalline area (memory state), to measure the change in optical transmission (read out), and to revert the same area to the amorphous state (erase).

These optical memories can also be operated in the so-called "reverse mode" (*J. Appl. Phys.*, **43**, 4688 ; 1972). An initial uniform crystalline state is obtained by heating the glass thin film to 90° C. The interaction with laser light produces an amorphous pattern which can be first "read" and then "erased" by heating once more to 90° C and cooling slowly. The advantages of this reverse mode of operation are that it gives a much faster "write time", allowing the rather slower reverse process to be used for "erasing".

It is very difficult at this stage of their development to predict the future for these devices, but undoubtedly they will be the subject of many more investigations, if only to resolve further the mechanisms involved in this phenomenon.

SAN ANDREAS FAULT

Pore Pressure and Creep

CREEP occurring in the central part of the San Andreas Fault—a right-lateral strike-slip fault running through California—can be related to changes in the pore pressure of water, as measured in a well 150 m deep, near Hollister. In this part of the fault the total creep of 1.2 cm yr^{-1} occurs as a series of small creep events.

Johnson, Kovach, Nur and Booker report (*J. Geophys. Res.*, **78**, 851 ; 1973) that both the maximum pore pressure and the offset in water level in the well are linearly related to the total creep in motions which occur within hours of anomalous changes in water level.

Three creep events, of magnitudes 4 mm, 3 mm and 2 mm, were associated with changes in water level of +5.6 cm, −4.1 cm and +3 cm, respectively. The water level started to change 4 h before the first event, 1.5 h after the second and 8 h before the third. The recorder used is sensitive only to within ±2 mm, insufficient for changes caused by Earth tides, for example, to have been monitored. The one to one correspondence — all anomalous water level changes were associated with fault movement—is particularly striking, and follows several recent experiments, notably in Colorado, where earthquakes have been produced by deliberate injection of water into the pores.

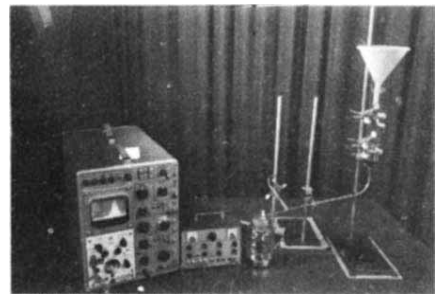
The success of those experiments has led to speculation that it might be possible to make the San Andreas Fault safe by drilling deep wells along the fault line and "locking" it by pumping water out. Selected regions of the fault could then be isolated and water pumped in to liberate the slip. Repeated small man-made earthquakes could ensure a relatively smooth slip without the sudden massive jerks which make the region so dangerous at present—or so the argument runs.

Johnson *et al.* point out that it is not possible, as yet, to say whether the transient pore pressure is produced by creep or the creep by changes in fluid levels. But deeper wells might be able to detect strains and pressure changes deeper in the Earth, monitoring pore pressure changes at different distances from the active region of the fault. If such monitoring proves effective, it may become possible to predict some of the behaviour of the active fault, or to understand the delicate balance of pore pressure and fault activity sufficiently well to permit experiments aimed at stabilizing the fault, by adding or removing water as required. That, however, is certainly a very long term prospect indeed.

Doppler Measurements of Metal Flow Velocities

ULTRASONIC Doppler velocimeters have been found useful in the measurement of fluid flow, for example blood flow. The technique depends, of course, on the presence of inhomogeneities in the fluid, from which the sound waves can be reflected. This would seem to make the technique unsuitable for studying liquid metal flow, but in the best traditions of "suck it and see" Fowlis has attempted to use such a velocimeter to measure the flow of both mercury and the liquid alloy NaK (47 per cent Na by weight, 53 per cent K). To his own surprise, he obtained a strong Doppler signal for both metals (see next Monday's *Nature Physical Science*, March 5).

The photograph shows the apparatus used, in which the Doppler shift of ultrasonic waves backscattered from the moving metal is recorded. The metal flows under gravity between two reservoirs along a tube in which the ultrasonic probe is mounted. The Doppler shift frequency range can be directly examined with the analyser and oscilloscope to the left.



The source of the backscatter is unknown, although Fowlis does point to dust particles or microscopic bubbles as possible candidates ; the important practical point, however, is that the system offers a cheap and apparently accurate means of measuring such flow, no matter how the effect works.

SELENOLOGY

Lunar Seismology

from a Correspondent

A CONFERENCE on geophysical and geochemical exploration of the Moon and planets was held at the Lunar Science Institute, Houston, Texas, on January 10 to 12. Although most of the contributions concerned the Earth-based research that had been pursued in recent years, some of the results presented were obtained from the Apollo programme, and there were even some very preliminary data from Apollo 17.

The first contributions, presented by Drs G. Latham and M. N. Toksöz (University of Texas, Galveston) and others, were concerned with the very interesting picture of seismicity that is emerging from the four Apollo seismograph stations now operating on the Moon. It seems that although the Moon is very seismically inactive by comparison with the Earth there is sufficient natural activity (arising from surface impacts and internal sources) for it to be possible to draw a firm outline of the Moon's internal seismic properties within the expected lifetime of this network. Judged as yet only by a very remarkable repetition of the waveforms of discrete events in the seismograms, some forty internal sources that must be very precisely located have now been identified. Only a few of these sources have been given a position within the Moon, but they all seem to be in a zone 600 to 1,000 km deep and there is some evidence that they tend to be immediately below the boundaries of the maria that are associated with mascons. In spite of the primitive state of the data there was talk of this seismicity being the result of mascons dropping back into the Moon—the whole process being triggered by the tidal effects of the Earth. In this connexion it is interesting that the results of laser altimetry from orbiting spacecraft presented by Dr W. R. Wollenhaupt (NASA, Houston) have shown that the sites of mascons are among the lowest places on the Moon's surface. From the study of a few impacts on the far side Dr Y. Nakamura (University of Texas, Galveston) reported that there seems to be a region of relatively high attenuation, with Q no greater than that of the Earth's upper mantle, below a depth of (approximately) 1,000 km.

Problems relating to the character and origin of lunar magnetism continued to attract attention. Information returned from the Apollo 15 and short-lived Apollo 16 sub-satellites travelling in near equatorial orbits, and presented by Dr P. J. Coleman (University of California, Los Angeles), shows that there is a highly structured lunar magnetic field (length scale tens to hundreds of kilometres) detectable at heights generally less than 100 km. This ties up with the few spot readings taken on the surface. As with the surface topography there seems to be a systematic difference in the character of this field on the near and far side, and there was continuing debate about the way such a field will influence the interaction of the solar wind throughout a synodic month. The cause of this field can be reasonably attributed to the observed remanence of the surface rocks, but the origin of that remains undecided. It only need be added that the discovery of high attenuation in an extensive central region of the Moon, combined with the celebrated lunar reverberation, makes the problem of demonstrating seismically the existence of a small dense core suitable as a site for a lunar dynamo that much more difficult. More results were presented by Dr T. Nagata (University of Tokyo) and Dr G. R. Olhoeft and his colleagues (NASA, Houston) on the electrical conductivity of lunar material under conditions relevant to the interpretation of very shallow and deep electromagnetic sounding of the Moon. Although in the earliest measurements on lunar rocks it seems that enough attention was paid to oxidation of the samples at high temperatures, the latest data do not affect the earlier result that the temperatures deep within the Moon may be no more than 1,000° C.

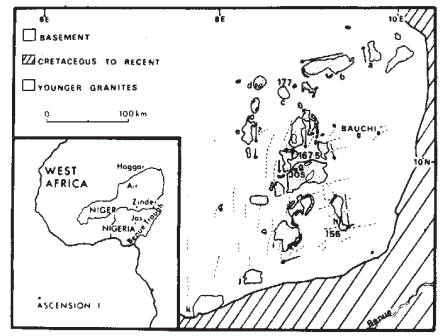
Interesting contributions were given by Dr C. R. Chapman (Planetary Science Institute, Tucson) and Dr T. B. McCord (MIT) on the analysis of the surface material of various bodies in the Solar System by spectrometric analysis of reflected light. There seems to be enough structure in some of the spectra to indicate differing compositions among the asteroids—some with no meteoritic equivalent. The absence of structure in the spectra of some of the largest asteroids is similar to that of basalt, and suggests that some of these may be chemically differentiated. There were also contributions on the interpretation of photographs of the Martian surface taken on the recent Mariner 9 flight.

Nigerian Thermal Plume Traces

IN next Monday's *Nature Physical Science* (March 5), van Breemen and Bowden report systematic geochronological data in respect of four Nigerian subvolcanic granites. These support the idea that igneous chains are thermal plume traces on moving lithospheric plates.

The non-orogenic granitic ring complexes of the Nigeria–Niger province, which form a narrow intrusive zone about 1,300 km long down longitude 9° E, fall into three geographical groups near Air, Zinder and Jos, respectively (see map). The central Nigerian complexes around Jos have previously been taken to be close to 160 million years old. The new, more detailed rubidium-strontium studies of van Breemen and Bowden, however, give a geographical pattern of ages ranging from 117 m.y. for the Liruei complex (*c* on the map), through 167.5 m.y. for the Amo complex (*f*) to 156 m.y. for the Pankshin complex (*h*). If this age range is taken to be the result of a plume trace, there is a clear implication here that at the relevant time Africa drifted northwards at a rate of about 0.76 cm a year—a conclusion which contradicts the suggestion from Burke and Wilson (*Nature*, **239**, 387 ; 1972) that Africa underwent a Mesozoic standstill.

Van Breemen and Bowden then discuss the consequences of the assumption that the other Nigeria–Niger granite complexes reflect plume traces. For example, the rate of 0.76 cm per year extrapolated to northern Air gives an age of about 300 m.y., which agrees with unpublished potassium–argon age determinations, although extrapolation to other granites at Hoggar, further north, leads to a discrepancy of about 200 m.y. between predicted and observed ages. Another feature of the Jos area granites which requires explanation is the two different trends represented by *a-d* and *i-k*, on the one hand, and *c-h* on the other. Van Breemen and Bowden tentatively suggest that these changes in trend reflect changes in the direction of Africa's motion relative to the breakup of Pangea.



Some geologists have related the Nigerian granites to a plume now thought to lie beneath Ascension Island, although there is apparently no seamount chain linking the island to Nigeria. Van Breemen and Bowden end by showing that palaeomagnetic data, plate tectonics and the motion of Africa predicted from the postulated Nigeria–Ascension Island plume trace are consistent, suggesting that little polar wandering has occurred since the mid-Jurassic.

QUASISTELLAR OBJECTS

Are All QSOs in the Nuclei of Galaxies?

by our Cosmology Correspondent

"ALL quasars occur in the nuclei of giant galaxies"—or, at least, "the observations are consistent with the hypothesis", according to Jerome Kristian, of the Hale Observatories. Coming close on the heels of the recent report that the QSO redshift-magnitude relation agrees well with the idea that these objects are at the cosmological distances implied by a Doppler interpretation of the redshifts (see *Nature*, **241**, 506; 1973), this discovery seems to have brought QSOs back into the limelight of astronomy, after a period in which the centre of the stage has been held by objects closer to home, within our own Galaxy.

Kristian's study (*Astrophys. J. Lett.*, **179**, L61; 1973) starts from the often discussed similarity between N galaxies, Seyfert galaxies and QSOs. These objects not only have the same qualitative properties, in terms of spectra, colours and variability, but also show a quantitative gradation in activity. This has led to speculation, for example, that the objects might be members of an evolutionary chain in which QSOs are the forerunners of Seyfert galaxies which in turn evolve into N galaxies. That idea does not stand up too well if there is no evidence for luminosity evolution of QSOs as a class, which now seems to be the case (Bahcall and Hills, *Astrophys. J.*, **179**, 699; 1973). Instead, the idea that QSOs are events occurring in the nuclei of galaxies, like those observed in the nuclei of N and Seyfert galaxies, becomes attractive. In that case, QSOs could easily be so much brighter than the equivalent events in N and Seyfert galaxies that their light output masks the light from the galaxies in which they lie, so that the characteristic starlike image of a QSO is produced on photographic plates. This is the idea that Kristian set out to test, using direct photographs of QSOs in an attempt to detect galaxies surrounding them. The results he obtains are impressive.

In order for such a photographic search to be successful, the image produced by a QSO at the centre of a galaxy must be smaller than the image produced by the galaxy itself. The QSO image size depends only on brightness, because QSOs are essentially point sources, but the galaxy image size depends on the actual size of the galaxy and its distance from the observer. This provides enough flexibility to make a photographic search for QSOs at galactic nuclei—or rather, galaxies centred on QSOs—feasible.

Kristian has taken the QSO redshifts as straightforward distance indicators, and has used calibrations of image size against magnitude for the QSOs and of apparent size against redshift for the galaxies. The latter calibration was already available from Sandage's work on brightest members of galaxy clusters (*Astrophys. J.*, **173**, 485; 1972); the former calibration was made by Kristian using *V* plates obtained with the 200-inch telescope. QSOs are usually identified from the *Palomar Sky Survey*, which is based on plates obtained with the 48-inch Schmidt camera. Underlying galaxies are not usually found associated with QSOs on the Schmidt plates, and it now seems that this is simply because, at the distances implied by QSO redshifts, galaxies are, in most cases, too small to produce an image larger than the QSO image on 48-inch plates.

Kristian has restricted his survey to objects which "have at one time or another been called quasars, and for which 200-inch plates or other indications of an underlying galaxy are available"; this gives twenty-six objects for analysis. In regions of the Hubble diagram where galaxies should be easiest to detect there are few QSOs (four were studied by Kristian) "but all of those for which good plate material is available show an underlying galaxy". These galaxies all satisfy the requirements of N galaxies, although because of the historical accident of discovery some are known by other names.

At the other extreme, where galaxies which are centred on QSOs should be difficult to detect none is found, although there are fourteen QSOs which satisfy Kristian's criteria, and in intermediate regions of the Hubble diagram there is evidence that five out of eight QSOs are at the nuclei of galaxies. The apparent diameters of the galaxies are as would be expected for N galaxies and bright cluster galaxies, and there are, says Kristian, also several cases in which the QSO is a little displaced from the centre of the galaxy rather than coincident with the nucleus. The evidence is impressive, but, of course, some QSOs may still not be at galactic centres.

Spin, Torsion and Gravitational Singularities

THE big-bang model of the Universe contains a singularity which is interpreted as the beginning. This is unsatisfactory in one way because the details of physical processes near to singularities are not well understood. It is, however, clear that quantum effects become important at high densities, although how to incorporate these effects into gravitational theory is an open question.

Oscillating models of the Universe also collapse periodically into singularities. The most obvious explanation is their very high symmetry and it would seem that the periodic collapse would be avoided if the model were slightly perturbed, allowing the incoming particles of matter to miss each other. Calculations by Penrose, Geroch and Hawking have, however, shown that this is not the case and that singularity is an intrinsic feature of general relativity which occurs under quite wide conditions subject only to very general and reasonable energy conditions.

General relativity therefore needs to be modified if singularities are to be avoided. Such a modification was suggested by Cartan and by Sciama in 1958 and incorporates an asymmetrical connexion to provide a model of spin. The cosmological consequences of this idea are worked out in next Monday's *Nature Physical Science* (March 5) by Trautman. In his communication the field equations of general relativity are modified by the presence of terms describing the torsion of the geometry arising from the spin of particles. Recently Kopczynski constructed non-singular models, based on the resulting modification of the Friedmann equations describing the expansion of the Universe according to the big-bang model. It turns out that there is a minimum radius for the 10^{80} particles of which the Universe is thought to consist. This radius is about 1 cm—quite large enough to avoid the difficulty of singularity, but small enough to have a negligible effect on the subsequent development.

Trautman considers the possibility that the spins were correlated in the hottest stage of the development of the Universe; the cosmic magnetic field might have played a significant part in this context. Trautman's is not a complete model, however, in that it neglects the magnetic field energy. Pressure is also ignored in the preliminary calculation.

Trautman also mentions the possibility of avoiding a singularity in a closed cosmological model. It is interesting to note the similarity of his modified Friedmann equations to those proposed some years ago in a continuous creation model of an oscillating universe by Hoyle and Narlikar.

T and B Lymphocytes and Immune Responses

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The recognition of two distinct classes of lymphocytes has been a turning point in immunology. Immunological models and tools may help to provide the answers to many biological problems.

IMMUNOLOGY has become an exciting science of its own. Nonetheless, what is being learned about lymphocytes and the immune responses that they mediate has important implications for medicine and other branches of biology. Unfortunately, the private language of immunology has made it difficult for non-immunologists to join in the excitement. This article attempts to review what is known in general terms about the cellular basis of immunity. (For a more detailed review of lymphocytes and their roles in immune responses, see ref. 1.)

Immunology is concerned with the specific responses an animal makes when foreign materials (antigens or immunogens) are introduced into its body. Such immune responses are made by all vertebrates and consist of the production of specific immunoglobulin protein molecules (antibodies) and/or specifically reactive cells, both of which can circulate in the blood and react specifically with antigen. As a result of this reaction, the foreign material may be inactivated (for example, bacterial toxins), killed (for example, infecting organisms or transplanted cells) and/or phagocytosed by cells of the reticuloendothelial system. On the other hand, in some cases, such immune responses may have deleterious effects on the host, such as in hypersensitivity reactions (hayfever and drug allergy, for example), where antigen reacting with antibody fixed to basophils and mast cells causes the release of histamine and other pharmacological mediators of inflammation. In general, immune responses which can be transferred to another animal by means of serum from a sensitized donor (containing antibody) are termed humoral immune (or antibody) responses, whereas those that can be transferred by sensitized cells but not by serum are called cell-mediated immune responses.

While immunochemists were unravelling the structure of antibody in the 1950s and early 1960s, cellular immunologists were demonstating that lymphocytes are the principal cells involved in immune reactions. The most convincing experiments were those showing that relatively pure populations of rat lymphocytes obtained from the chief lymphatic vessel, the thoracic duct, could transfer both cellular and humoral immunity to irradiated rats, which could not respond immunologically themselves as their lymphocytes had been killed by the radiation (reviewed in ref. 2). In addition, depleting animals of lymphocytes by prolonged drainage of the thoracic duct was found to impair their immune responsiveness[2]. Thus lymphocytes, whose origins and functions had been a mystery for so long, were established as "immunocompetent" cells.

It was soon realized that lymphocytes are not a homogeneous population. Several lines of evidence suggested that there are two distinct types of immunocompetent lymphocytes: one which requires the thymus gland for development and is responsible for cell-mediated immunity and another which develops independently of the thymus and mediates humoral antibody responses. The evidence came from studies in birds, rodents and man in the 1960s. In birds[3,4] and rodents[5] it was found that removing the thymus from an embryo or newborn markedly impaired the cell-mediated immune responses of the animals when they grew up, but had much less effect on humoral immunity. On the other hand, removal at hatching of the bursa of Fabricius[3,4], a cloacal lymphoid organ unique to birds, impaired the bird's ability to make antibody, but had little effect on cell-mediated immunity. Investigations of patients with immunological deficiency diseases also showed that humoral and cell-mediated immunity could be separately affected (reviewed in ref. 6): patients with Bruton-type congenital agammaglobulinaemia could not make antibody and were deficient in lymphoid cells producing antibody, but had normal cell-mediated immunity, whereas children with congenitally hypoplastic thymus glands (for example, Di George's syndrome) had markedly impaired cell-mediated immunity but could make relatively normal amounts of antibody in response to some antigens.

In the past few years the two-lymphocyte model of immunity has been firmly established (at least in birds and mammals), with two "central" lymphoid organs—the bursa, or its mammalian equivalent (still unidentified), and the thymus—producing lymphocytes independently of antigen, and seeding them out to the "peripheral" lymphoid organs (that is, lymph nodes, spleen and gut-associated lymphoid tissues) where they await contact with antigen which will induce them to differentiate into "effector" cells (see later). In the peripheral lymphoid tissues the lymphocytes derived from thymus are referred to as T cells, while those derived from the bursa in birds, or its equivalent in mammals, are called B cells[7].

Phylogeny

Until recently it was thought that specific immune responses were confined to vertebrates. There is now evidence, however, that some invertebrates, such as annelids and tunicates, can reject foreign tissues and that these primitive immunological responses can display specificity and possibly short-term memory[8] (that is, an increased and/or faster response on second exposure to the same antigens). These reactions are mediated by macrophage-like cells (coelomocytes) and possibly by soluble effector molecules having relatively little specificity[8]. As there is no evidence that invertebrates have lymphocytes or immunoglobulins, it seems likely that specific cellular immunity evolved before the appearance of these two principal mediators of vertebrate immunity.

All vertebrates have lymphocytes and probably thymus tissue (at least at some stage in their development) and are capable of producing antibody and cell-mediated immune responses[8]. Lower vertebrates (lampreys and hagfish, for example) have little organized lymphoid tissue and can produce only one class (IgM-like) of antibody. Rudimentary lymph node-like structures are first found in Amphibia which make two classes of antibody. Birds are the first vertebrates in which a clear dichotomy of the lymphoid system has been established, and are unique in having two discrete central lymphoid organs, thymus and bursa, producing T and B lymphocytes respectively. Mammals have abundant and highly organized lymphoid tissues, can elaborate a variety of different classes of antibody (such as IgG, IgM, IgA, IgE, IgD in man) and have distinct T and B lymphocyte populations, although the site of B cell development is still uncertain. It is not known whether vertebrates below birds have separate classes of T and B cells.

Development of T Lymphocytes

In most animals, lymphocytes first appear in the foetal thymus. The thymus anlage is composed of epithelial cells and is derived from the third and fourth pharyngeal pouches. Although in the past it had been suggested that thymus lymphocytes (thymocytes) develop from thymus epithelial cells, experiments in chickens and mice have clearly established that haemopoietic stem cells from foetal yolk sac and liver migrate into the thymus anlage and there proliferate and differentiate into thymus lymphocytes, presumably under the inductive influence of the thymus epithelium[9]. In mice (gestation 20 days) the first stem cells, which seem to be large basophilic blast-like cells, arrive in the thymus around day 11, and the first small lymphocytes are seen by day 15 or 16 of embryonic life[9]. Using radioactive[10], chromosome[5,11] and surface antigenic[9] markers, it has been shown that lymphocytes migrate from thymus to peripheral lymphoid tissues to make up the T lymphocyte population. Although this begins just before birth in mice, most of the seeding occurs in the first week of life[9]. Therefore, if the thymus is removed in the first days of life the mouse will grow up with a marked deficiency of T cells and thus impaired cell-mediated immunity, whereas thymectomy done later in life has much less effect[5]. In adult animals, stem cells from bone marrow migrate to thymus, and thymus lymphocytes continue to seed to the periphery, but these processes take place at a much reduced rate by comparison with the foetus and newborn[5,11].

Most thymus lymphocytes are immunologically incompetent (that is, they cannot respond to antigen) and differ in other ways from peripheral T cells, suggesting that there is another differentiation step from thymocyte to T lymphocyte. Recently it has been demonstrated that there is a small subpopulation (~2 to 5%) of thymus cells, located in the thymus medulla, which is immunologically competent and has most of the properties of peripheral T lymphocytes[9,12,13]. This suggests that the second differentiation step may occur within the thymus and that T cell development may be visualized as stem cell→thymocyte→"mature" thymus lymphocyte→peripheral T lymphocyte (Fig. 1). This scheme is almost certainly an oversimplification, however, for there is some evidence that cells may leave the thymus at varying stages of maturation, or perhaps as distinct cell lines, giving rise to subpopulations of peripheral T cells with different properties and functions[13]. In addition, the role of putative thymus humoral factors or hormones (thymosin, for example) is still unclear, although there is evidence that they probably do not induce stem cells to differentiate to lymphocytes outside the thymus, but may influence peripheral T cells in some way[14].

Development of B Lymphocytes

In birds, B cell development is dependent on the bursa of Fabricius which arises as a sac-like evagination of the dorsal wall of the cloaca on day 5. Chromosome marker studies have shown that stem cells (morphologically identical to those seen in the foetal thymus) begin to migrate from yolk sac to the bursa around days 12 to 13 and there differentiate to lymphocytes within 1 or 2 days[9]. By day 14, bursa lymphocytes with IgM on their surface can be seen, and bursa lymphocytes bearing IgG are seen a few days later[15]. The migration of bursal lymphocytes to peripheral lymphoid tissues has been demonstrated by isotope labelling experiments. Embryonic bursectomy results in marked depletion of peripheral B lymphocytes and a marked impairment in antibody (that is, immunoglobulin) production[15]. Recently it has been found that injecting anti-μ antibody (that is, specific for the heavy chains of IgM) before hatching, combined with neonatal bursectomy, suppresses later production of IgG as well as IgM[15]. This suggests that even B cells that will eventually produce IgG initially express IgM on their surface, and is strong evidence for an IgM→IgG switch within individual B cells. Whether this switch is driven by antigen, as suggested by experiments in mice[16], or occurs independently of antigen stimulation, as suggested by experiments in chickens[15], is unsettled.

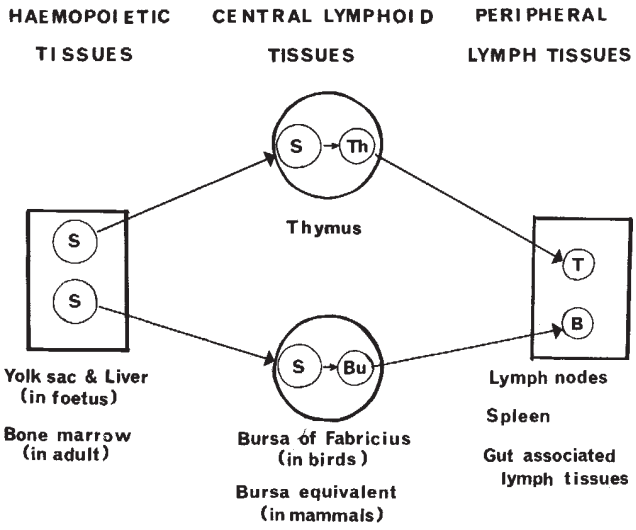

Fig. 1 Diagrammatic (and oversimplified—see text) representation of T and B lymphocyte development showing migration of stem cells (S) to thymus and bursa where they differentiate to thymus (Th) and bursal (Bu) lymphocytes, some of which migrate to the peripheral lymphoid tissues as T and B lymphocytes respectively.

In mammals, it is still not clear where stem cells differentiate to B-type lymphocytes, although it is known not to be in the thymus. It has been suggested that gut-associated lymphoid tissues (like Peyer's patches, tonsils, appendix, and so on) may serve as "bursa-equivalent", but there is little evidence to support this. In rodents, at least, there is increasing evidence that lymphocytes are produced in large numbers in the haemopoietic tissues themselves[17] (that is, liver in embryos and bone marrow in adults) and it seems likely that these tissues not only supply the stem cells for both T and B cell populations but are also the sites where stem cells differentiate to B-type lymphocytes.

It is not clear at what stage stem cells are committed to becoming lymphocytes or to becoming T or B cells. The finding of multipotential haemopoietic stem cells (that is, cells capable of becoming any of the mature blood cell types, lymphoid or myeloid) in early mouse embryonic thymus[18] suggests that commitment may not occur until stem cells enter the microenvironment of the thymus or bursa (or bursa equivalent).

Distinctive Properties of T and B Lymphocytes

As resting T and B lymphocytes are morphologically indistinguishable and are found together in all peripheral lymphoid tissue, it has been essential to find ways of distinguishing and separating them in order to study their individual properties. The demonstration of important surface differences between them has been particularly useful in this regard. Some of these surface differences can be recognized by antibody[19]. For example, the θ alloantigen (defined by alloantibody made in one strain of mouse against thymocytes of another strain) is present on mouse thymocytes and T cells, but absent from B lymphocytes, and this has proved to be a convenient surface marker for T cells in mice[19]. On the other hand, readily demonstrable surface immunoglobulin (Ig) (refs. 20, 21) and the heteroantigen, "mouse-specific B lymphocyte antigen" (MBLA) (ref. 19)—defined by hetero-antibody made in rabbits against mouse B cells—can serve as B cell markers. With antisera reacting specifically with the surface of one or other lymphocyte

type, either cell population can be killed in the presence of complement, and thus eliminated from a cell suspension. Alternatively, one can use antibody on digestible solid-phase immunoabsorbents[22], or fluoresceinated antibody and fluorescence-activated electronic cell sorting[23], to purify either type of cell. In addition to surface antigenic differences between T and B cells, the latter can bind antibody-antigen-complement complexes by means of surface complement receptors[24], and antibody-antigen complexes by means of receptors for the Fc part of complexed Ig[25]; resting T cells do not have these receptors. The functions of Fc and complement receptors on B cells are unknown, but it has been suggested that they may be important in antigen localization in the lymphoid tissues, in B cell activation by antigen and/or in putative killing by B cells of target cells coated with antibody.

Most T lymphocytes continuously recirculate between blood and lymph, passing out of the blood through specialized post-capillary venules in lymph nodes and Peyer's patches, passing through the substance of the lymphoid tissues and entering the efferent lymph; they then re-enter the bloodstream by way of the thoracic duct[2,5]. Although most B lymphocytes seem not to recirculate, some apparently do, but through different areas of the lymphoid tissues and with a slower transit time than T cells[26]. In the peripheral lymphoid tissues, T and B cells are found in more or less separate areas, the so-called thymus-dependent areas (periarteriolar sheath of spleen, paracortex of lymph nodes, and interfollicular areas of gastrointestinal lymphoid tissues) and thymus-independent areas (lymph follicles and peripheral regions of splenic white pulp, follicles and medulla of lymph nodes and follicles of gastrointestinal lymphoid tissues) respectively[27]. When radiolabelled T or B cells are injected into an animal, they migrate specifically to their respective areas[27]. Although both T and B lymphocyte populations are heterogeneous[1], T cells have a longer generation time[28] on average and are slightly larger[29], more dense[24], less adherent[24] (to various materials such as glass, plastic, nylon, and so on) and more negatively charged than B cells[30]. In addition, T lymphocytes are preferentially depleted by anti-lymphocyte serum[31] (which acts principally on recirculating cells), but in general are less sensitive to cytotoxic drugs (for example, cyclophosphamide[32]), corticosteroids[33] and irradiation[34]. T and B cells also differ in their *in vitro* responses to a variety of "mitogens", such as plant extracts (phytomitogens), bacterial products (like endotoxin) or antibodies to lymphocyte surface antigens, which stimulate a relatively large proportion of T and/or B lymphocytes to divide and differentiate into blast cells. Although pokeweed stimulates both T and B cell proliferation, concanavallin A (Con A), phytohaemagglutinin (PHA) and lentil stimulate only T cells, and lipopolysaccharides (for example, *E. coli* endotoxin) and anti-Ig sera stimulate only B cells[35]. It is of interest that although soluble Con A and PHA selectively activate T cells, they bind equally well to B cells, and if covalently linked to solid-phase materials they stimulate B cell proliferation[35]. Mitogen stimulation of lymphocytes is being intensively studied as a possible model of lymphocyte activation by specific antigen. These studies have made it clear that there is more to lymphocyte activation than simple binding of ligand to surface receptors.

Antigen Recognition and Specific Lymphocyte Receptors

The central dogma of immunology is the clonal selection hypothesis which suggests that at some time in ontogeny and independently of antigen, individual lymphocytes (or clones of lymphocytes) become committed to responding to one, or a relatively small number of antigens; they express this commitment through antigen-specific receptors on their surface. Thus, when an antigen is introduced into the body it selects out those lymphocytes which already have receptors for the antigen on their surface; the interaction of antigen with receptors initiates the activation of the specific cells. There is now an impressive body of evidence supporting the clonal selection hypothesis for both T and B lymphocytes. Thus T and B cells have been shown to bind antigen to their surface[36] (although it has been more difficult to demonstrate T cells binding antigen than B cells) and in general only a small proportion of lymphocytes (~ 1 in 10^4 to 10^5 in unimmunized animals) bind any one antigen. Furthermore, if lymphocytes are exposed to a highly radioactive antigen, both T and B cell responses to that antigen can be selectively abolished, while responses to other antigens are unaffected[37]. Similarly, B cells capable of responding to a particular antigen specifically adhere to glass beads coated with the antigen and can thus be specifically removed from a cell suspension[38]. Although T cells tend not to adhere under these conditions[38] for reasons that are unclear, T cells responsive to cell surface alloantigens can be selectively removed in cell monolayers bearing the specific alloantigens[39].

In 1900, Ehrlich proposed that cells producing antitoxins (now known to be B cells) had antitoxin molecules as receptors on their surface. The more recent version of the receptor hypothesis suggests that B lymphocytes have antibody molecules (that is, Ig) as receptors for antigen, which, at least in their combining sites, are identical to the antibody which the cell or its progeny will eventually secrete. There is now good evidence for this view, in that B cells have been shown to have Ig molecules on their surface ($\sim 10^4$ to 10^5 a cell) (refs. 20, 40) and anti-Ig antibody inhibits their ability to bind or respond to antigens (reviewed in ref. 1). There is also increasing evidence that the antigen-specificity of receptors and secreted antibody are the same for any one B lymphocyte clone[41,42]. The Ig class of the receptors and that of the ultimately secreted antibody may not, however, always be the same, for B cell precursors of some IgG secretory cells seem to have IgM receptors[15,16]. As different antibody classes (for example, IgG and IgM) seem to be able to share the same specificity (that is different Ig constant regions can be associated with identical Ig variable regions[43]) an IgM→IgG switch within a single clone need not imply a switch in specificity. In mice, at least, there is some evidence that most virgin B cells have IgM receptors (in its 7–8S monomeric form[44]) which may switch class after a primary exposure to antigen[16]. The more fundamental question of how antibody diversity is generated, that is how an animal develops the ability to synthesize such a large number of different Ig molecules (receptors and secreted antibodies) is still being debated. Germ-line theories, which suggest that one is born with a large number of variable region Ig genes, are competing with various somatic theories, which postulate that one is born with few variable region Ig genes and that some somatic process (for example, mutation or recombination) creates a large number.

The chemical nature of receptors on T cells is probably the most controversial issue in cellular immunology at present. The simplest and most logical view, that only antibody can recognize antigen and that all antigen-specific receptors must be Ig, has been challenged by the failure of many investigators to demonstrate Ig directly on the surface of T cells, or to inhibit various T cell responses with anti-Ig sera. Indeed, there is now growing support for the idea that surface components other than classical immunoglobulin may play an important role in T cell recognition of and/or response to at least some antigens. The principal candidates for such T cell "receptors" are the products of the immune response (Ir) genes that are genetically linked to the chief histocompatibility loci[45]. These Ir genes influence T cell responses to a variety of antigens[46]. The exquisite specificity of T cell responses, which resembles very closely the specificity of antibody and B cell recognition[47], taken together with the various (but still controversial) demonstrations of Ig on T cells (reviewed in ref. 1), makes one reluctant, however, to give up the idea that T cells have Ig receptors. It is possible that T cells (and possibly B cells) have at least two "recognition" systems, one involving Ig and another mediated by Ir gene products, the

general importance of each varying depending on the antigen, the response and/or the subclass of T cell. The putative non-Ig recognition system could be analogous to the primitive recognition of foreignness seen in invertebrates.

Functions of T and B Cells

When an antigen combines with its corresponding receptors on a T or B lymphocyte, one of at least three things can happen to the lymphocyte: first, it may be stimulated to divide and differentiate to become an effector cell in some type of immune response (that is, it is induced to respond immunologically); second, it may become immunologically tolerant or paralysed, so that it will not be able to respond the next time antigen is given; it is not known if such cells are killed or simply inactivated in some way; third, it may be unaffected by the encounter. In addition, if the animal makes an immune response to the antigen, on subsequent exposure to the same antigen, it will usually give a faster, greater and sometimes qualitatively different response. This altered state of immune reactivity to a specific antigen is called immunological memory. It is likely that memory involves both clonal expansion (that is, division of virgin lymphocytes to give an increased number of cells able to respond on second exposure) and differentiation of virgin cells to memory cells[1], but it is unclear whether memory cells are simply retired effector cells, cells at an earlier stage of differentiation than effector cells, or are derived by differentiation along a separate memory pathway.

The "decision" of an individual lymphocyte on encounter with antigen—whether to "turn-on", "turn-off" or ignore—depends largely on the nature and concentration of the antigen, and upon complex interactions with other lymphocytes and with macrophages. Although most immunogens can stimulate both T and B cell responses, some, particularly those with repeating identical determinants and which are poorly catabolized—the so-called "thymus-independent antigens" (for example, pneumococcal polysaccharide, *E. coli* endotoxin, polyvinylpyrrolidone)—chiefly stimulate B cells (reviewed in ref. 1), whereas others preferentially activate T cells[48]. In general, T cells respond to lower concentrations of antigen than do B cells, and although T cells may be paralysed at very low and very high concentrations of antigen (low and high zones of tolerance respectively) B cells seem to be paralysed only at high antigen concentrations[49]. The way in which the antigen-receptor interaction signals a lymphocyte is unknown, although it probably involves allosteric changes and/or redistribution (for example, aggregation into patches or localization over one pole—cap formation[50]) of the membrane-bound receptors.

The most important differences between T and B cells concern their different functions in immune responses. When B cells are activated by antigen they divide and differentiate into blast cells with abundant endoplasmic reticulum, and some go on to become plasma cells. These cells remain in the lymphoid tissues for the most part and secrete large amounts of antibody which circulates in the blood. Individual antibody-secreting cells can be detected by a variety of techniques, the most common being the plaque-forming cell assay, in which anti-erythrocyte antibody released from single B cells lyses erythrocytes in their immediate environment in the presence of complement. Antibodies, in conjunction with various accessory cells (macrophages, mast cells and basophils, for example) and particular serum enzymes (complement components, for example), are responsible for a variety of hypersensitivity reactions and protective immunity against many pathogenic organisms. In addition, antibody serves to regulate the function of both T and B cells, inhibiting their responses by competing with lymphocyte receptors for the antigenic determinants, diverting antigen from the lymphoid tissues or by forming tolerogenic antibody-antigen complexes[51], and enhancing responses by localizing antigen to appropriate lymphoid tissues or perhaps forming immunogenic antibody-antigen complexes. It is also possible (but not established) that B cells themselves play a direct part in transporting antigen (perhaps as antigen-antibody ± complement complexes adhering to Fc or complement receptors on B cells) and/or in killing target cells with coated antibody[52].

When T cells are activated by antigen, they proliferate and differentiate to become blast cells, but they do not develop significant amounts of endoplasmic reticulum and do not become antibody-secreting cells. They do, however, secrete a variety of non-antigen-specific factors ("lymphokines") such as migration inhibition factors (MIF), chemotactic factors, cytotoxic factors and mitogenic factors, at least some of which presumably play a role in cell-mediated immune responses, for which T cells are primarily responsible[53]. The precise chemical nature of these factors, the relationship between them, their significance and mechanisms of action are, however, incompletely understood. Cell-mediated immune responses include delayed hypersensitivity, contact sensitivity, rejection of foreign tissues, graft *versus* host responses (where injected foreign T lymphocytes respond against the antigens of the recipient, often resulting in recipient death) and immunity to various microbes. In all of these responses, T cells enlist the help of macrophages (probably through the secretion of lymphokines). The latter are usually the predominant cells at the site of these reactions[54]. T cells can also be demonstrated to respond to antigen *in vitro* by dividing, secreting lymphokines, killing target cells, or supporting viral replication (reviewed in ref. 1). Whether T cells themselves can directly kill target cells, or do so only by activating other cells (such as macrophages) is still controversial, although there is increasing evidence that they can become "killer cells" under some circumstances[55].

Although T cells do not themselves secret antibody in the usual sense, it is now known that they play an important role in helping B cells to make antibody responses to most immunogens. Thus, in these responses T cells are referred to as "helper" cells, and B cells as "antibody-forming precursor" cells. The first direct evidence for such T-B cell cooperation was provided in 1966 by the observation that irradiated mice given both thymus cells and bone marrow cells made a far greater antibody response to sheep erythrocytes (SRBC) than recipients of either thymocytes or bone marrow cells alone[56]. Subsequently it was shown that all of the antibody-secreting cells (that is, those making anti-SRBC antibody) in this type of experiment came from the bone marrow inoculum[57]. Independent studies with chemically defined antigens showed that T-B cell cooperation in antibody responses involved T cells responding to one antigenic determinant on an immunogen and helping B cells to respond to different determinants on the same immunogen[58]. Although it is clear that cooperation is usually mediated by such an "antigen bridge" between T cell and B cell receptors, it is still uncertain whether the bridge is between T and B cells themselves, or between shed T cell receptors (perhaps taken up on the surface of macrophages) and B cells, and whether the bridge serves to "present" antigen to B cells in a particularly immunogenic form (concentrated and multivalent, for example) or to bring B cells close to T cells or a third party cell (such as macrophage) so that a non-specific, short-range factor (for example, chemical mediator or membrane-membrane interaction) can operate between them (Fig. 2). Although it has been shown that T cells can secrete non-specific factors which can enhance B cell responses[59], their role in normal T-B cell cooperation is still uncertain. There is recent evidence that, in some *in vitro* responses at least, cooperation may involve the release by T cells of antigen-specific IgM-like factors (? receptors) complexed with antigen, which are subsequently taken up on macrophages[60].

There are antigens ("thymus-independent antigens") which seem to be able to stimulate at least some B cell clones to secrete IgM antibody without the help of T cells (reviewed in ref. 1), suggesting that T-B cell collaboration is not always essential for antibody production. Nonetheless, the discovery

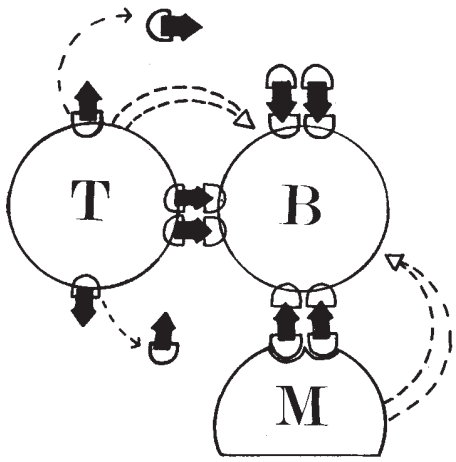

Fig. 2 Possible mechanisms of T-B cell collaboration in humoral antibody responses. The "antigen bridge" (→) between T and B cell receptors could serve to: (i) present antigen to B cells on the surface of T cells or as a matrix of released T cell receptors complexed with antigen, either free or on the surface of third party cells such as macrophages, or (ii) bring B cells together with T cells or a third party cell so that a short-range factor can operate between them.

that T cells cooperate with B cells in humoral immunity has been an important advance and has explained the previous paradox of impaired antibody responses in T cell deficient animals. There is recent indirect evidence that T cells can inhibit B cell activity as well as enhance it[61], and that they can enhance[62] and inhibit[63] the functioning of other T cells. It is not known if these interactions involve antigen bridging between the receptors of the interacting cells. Taken together with the enhancing and inhibiting effects of secreted antibody on both T and B cell functions, a picture is emerging of a highly complex and finely controlled immune system, with each type of cell and response modulating the others.

Way Ahead

With the recognition that there are two distinct classes of lymphocytes with different origins, properties and immunological functions which modulate each other's activities, the door has opened to a new era of immunology. The resulting insight into the functioning of the immune system in health and disease has paved the way for rational attempts to manipulate selectively the different cell types and their various responses for the benefit of patients with infection, autoimmune disease, cancer, immune deficiency states, and organ grafts. And present day immunology provides a number of readily accessible models and powerful tools for studying a variety of biological problems, including differentiation, genetic control, cell interactions, and membrane receptor-ligand interactions.

I am grateful to M. F. Greaves, N. A. Mitchison and J. J. T. Owen for helpful discussion. The bibliography, which is incomplete, is meant only as an arbitrary way into the relevant literature.

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Multiple Universes

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Professor Gold considers the properties of a closed or nearly closed universe in which there are substantial variations of mean density on a large scale, so that close "sub-universes" could exist within it.

MOST cosmological discussion assumes that the Universe, although locally obviously non-uniform in its content, tends to uniformity on a large scale. In particular it is assumed that on the scale of the "radius of the Universe" R_0 (defined by setting $V=c$ in the Hubble expansion law $D=V/H$, where V is the velocity of objects observed at the distance D and H is Hubble's constant) sufficient uniformity is reached for the principal conclusions of geometry to depend on a single large scale radius of curvature only.

Modern observations of distant parts of the Universe do not make a clear case for such uniformity. A number of seemingly strange non-uniformities have been pointed out, such as superclusters of galaxies[1] or patterns of quasars[2,3]. No observations seem to demonstrate that a smooth distribution of matter does indeed exist on the largest, cosmological scale of distances.

Sandage *et al.*[4] have presented evidence that no large dynamical effects can be attributed to inhomogeneous mass distributions on the intermediate distance scale accessible by optical observations of ordinary galaxies. On the other hand, the evidence of quasars, which refers to a much larger distance scale if one assumes that universal expansion is the chief cause of their redshifts, is still entirely confusing (see, for example, Rees[5]). It is therefore appropriate to consider the consequences of the existence of non-uniformity on the large scale also. Time constants for gravitational contraction of matter unresisted by physical pressure are given by $(G\rho)^{-\frac{1}{2}}$ (where G is the gravitational constant and ρ is the density of the contracting region) and are independent of the scale. Thus a large region can collapse on this basis just as fast as a small one. The condition for closure of our Universe $\frac{2GM_0}{R_0}=c^2$ can be written as

$$\frac{8\pi G R_0^2 \rho_0}{3} = c^2$$

or, leaving out all small numerical factors, as

$$(G\rho_0)^{-\frac{1}{2}} = \frac{R_0}{c}$$

$(G\rho_0)^{-\frac{1}{2}}$ is the time constant for gravitational collapse for a region of any scale but of density equal to the mean, and R_0/c is the "age of the Universe" defined by the expansion. Apart from small factors which are different in different cosmological models the two times are the same. Thus a universe of such density that it is closed or nearly closed is one in which there has been enough time available to generate great density variations on any scale. The general expansion and physical pressures would oppose the growth of such inhomogeneities. The effect of expansion as a stabilizing influence is different in different cosmological models; physical pressure is always least important on the largest scale. There is no general argument that the large scales should have been protected from the growth of gravitational condensations when it is clear that smaller scales were not.

The mass density existing in large spaces is observationally a very poorly known quantity. Obviously the assumption that all mass is in stars that are luminous is a poor one with no real justification. Even the assumptions that mass is chiefly in galaxies, and that galaxies emit light in proportion to their mass, are very questionable. The dynamical estimates seem to lead in each case to a large amount of unseen mass; for example the dispersion of velocities seen in clusters of galaxies is very much greater than the values that would be given by the virial theorem for masses estimated by means of the assumptions mentioned above.

Unseen mass can exist in several forms that would have escaped present means of detection. Ionized hydrogen can occur in comparatively large amounts in intergalactic space; cold disks of rotating condensed matter may be as common as stars; black holes of stellar masses may also be common; gas galaxies, in which star formation has not proceeded very far, may be members of clusters. Thus the mean density in the

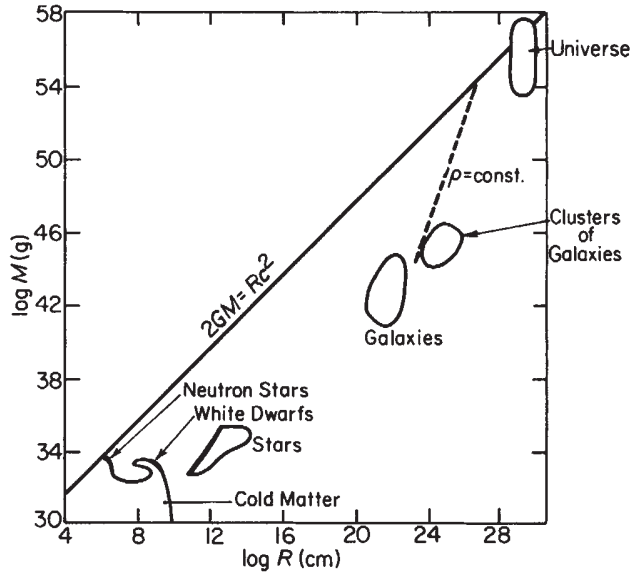

Fig. 1 Radius (R) against mass (M) for objects in the Universe. The lower right half contains known objects; the diagonal line represents the gravitational singularity. - - -, Constant density line showing where the largest clusterings of matter might be situated, up to the singularity.

Universe is at least 10^{-33} g cm^{-3}, as judged from the starlight emitted by galaxies, but it may be more. A value in the vicinity of 10^{-30} g cm^{-3} is possible, and that, for modern estimates of the expansion constant, would make the Universe closed within the radius R_0; in fact, most cosmological discussions assume a density of this order.

It is of interest then to consider the geometrical properties of a universe which is closed or nearly closed within R_0, but in which there are substantial variations in mean density even on a scale comparable with R_0. In such a universe subunits may have the mass necessary to make them closed in turn. The requirement for that would be that their density is greater than the mean by a factor $\left(\frac{R_0}{R}\right)^2$, where R $(< R_0)$ is the radius of such a region. Therefore progressively smaller irregularities of density are sufficient to cause closed subunits as one approaches the scale of the "radius of the universe" R_0.

Black holes of stellar or galactic masses have been discussed as being possible members of our Universe. But there the factor $\left(\frac{R_0}{R}\right)^2$ is very large; high densities have to have been built up and the complex physical processes leading to such contractions of matter need to be discussed. On a large scale the densities implied by closure are low, and not of a kind that would be associated with any particular set of physical processes, but merely with the statistics of the large scale distribution of matter. If, as larger and larger scales are examined, one again encounters excursions of the density from the cosmological mean approaching the factor $\left(\frac{R_0}{R}\right)^2$, where R is the scale examined, then one must suspect that singularities on such a scale may also exist in our Universe. Clusters of galaxies have densities which, if estimated on the basis of the velocity dispersion and the virial theorem, are indeed greater than that necessary to generate singularities on a scale of $\frac{1}{10}R_0$; thus if density variations of this or even somewhat smaller magnitudes persist to large scales, they would be singularities and therefore would not be seen as large clusters.

Such objects would not be readily identifiable. In our geometrical picture they would appear as points, or, if insufficient time has been available for the completion of the distortion, whose last phase is infinitely slow in our time frame, they would appear as very small areas of great redshift. There is no reason for associating any great luminosity with them. Internally they may be universes smaller than ours only by a small factor. The particular space open to us may in turn be a closed subunit in another slightly larger universe. Indeed, if we are embedded in a space having more than a certain dispersion of density, we would have to regard it as coincidental if our accessible space, our Universe, was the first (in ascending order of size) that possessed the density for closure. If not, then other "universes" would be part of ours, just as ours might be a component of a larger one. There would be a system of "nesting universes" in which each is perhaps only slightly smaller than the one in which it nests.

There is, of course, no theoretical constraint preventing our discovery of massive singularities in our Universe; but, within the understanding of the movement of information in relativity theory, we would have no access to any information about any larger scale than that of our closed space.

Can present observations tell us whether large gravitational singularities exist in our Universe? The geometrical properties of our Universe would of course be severely affected if most of its mass were in the form of a small number of singularities. As the "seen" mass is only approximately 10^{-3} of that necessary for closure, these may, in the most extreme case, be as much as 10^3 times the "seen" mass in the form of large singularities. Space would then be extremely uneven both in directional and in temporal properties. Distant light sources would be seen in directions severely distorted by space curvature and the local density of any class of objects is then quite different from the density determined from our vantage point, without allowance for this distortion. Equally the frequency shifts of spectral lines would be severely changed by the gravitational redshift, and different for different large regions of observations.

Gravitational redshifts seemed inadequate as an explanation for observed redshifts when only mass concentrations on a galactic scale were assumed and any gradient of potential over the luminous region would widen the lines beyond the observed amount. There is no such difficulty if one assumes that there are large, cosmic scale singularities. In their vicinity galaxies, and even large clusters of galaxies, can be accommodated in a gravitational potential of very slight gradient only, but of a value very different from that in our locality; we would therefore observe such galaxies with a spectral shift due to this difference in gravitational potential, superimposed on any Doppler shift due to expansion. The observation of blueshifts would then be a possibility in principle, for we could observe galaxies in lower gravitational potentials than that of our neighbourhood. The general expansion may, however, make such an effect very improbable and result in the observation of redshifts only. The possibility of regional effects does, then, exist. There may be significant variations in the number per unit solid angle of objects seen within a certain range of values of the redshift. Such effects have been suspected[2,3], but their statistical significance is still not clear.

The universal background radiation is not expected to show any anisotropies as a result of distortions of space geometry. A uniform surface brightness remains uniform and unchanged when viewed through space refracting the rays in any manner whatever (this of course is required to avoid an infringement of the Second Law of Thermodynamics). Thus if the background radiation was set up initially as a uniform brightness, the subsequent development of gravitational distortions of space would not have any effect. The absorption of radiation by a singularity is an effect that could introduce an observable anisotropy, but the time scale of the development of this effect is not clear.

The observation of the apparent strengths of radio sources, without knowledge of distance or redshift, is concerned with the greatest number of objects and may therefore be most sensitive for the detection of severe space distortions. But here also there are severe limitations. A uniform distribution of sources in Euclidian space results in the relation $N = S^{-3/2}$ where N is the number of sources seen of an apparent strength exceeding S. The form of this relation is maintained when the region is viewed through a convergent or divergent lens, but the numbers are changed, although not very critically. Thus the isotropy of this type of observation can place a limit on space distortions, but only if it is certain that the contribution in each sample is dominated by intrinsically strong and distant sources rather than by intrinsically faint but nearer ones. The indication that this is so is derived from the appearance of a slightly steeper law in the relation between N and S than that for uniform Euclidian space, but observers are not yet in agreement as to the reality of this effect. Nevertheless it is clear that modern observations by radio and optical means will be able to give a limit to the extent of space distortions occurring in our Universe, and therefore it will be possible to answer the question whether there are other universes nesting within our observable space.

I thank Nordisk Institut for Teoretisk Atomfysik for hospitality.

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Intrusion, Extrusion and Metamorphism at Constructive Margins: Evidence from the Troodos Massif, Cyprus

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Evidence from the Troodos Massif for the origin of the ocean floor is discussed and a detailed model formulated.

RECENT proposals that the Troodos massif of Cyprus represents a subaerially exposed slice of oceanic lithosphere[1-4] have received wide acceptance. In some of these works[2,3] the principal units of the massif have been correlated with seismic layers 2, 3 and 4 of the ocean floor (Fig. 1).

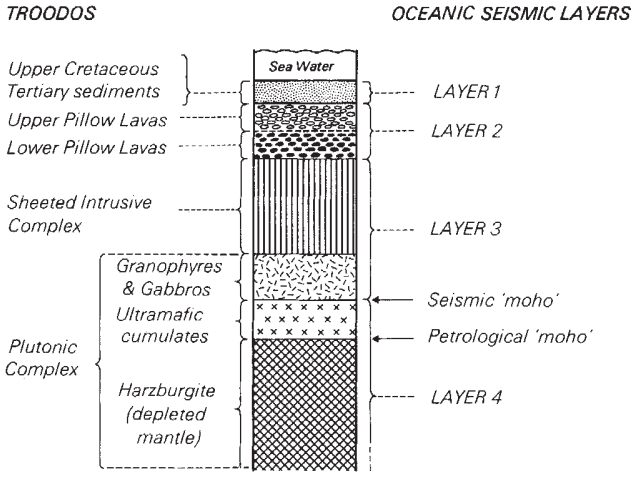


Fig. 1 Correlation between Troodos units and oceanic seismic layers.

The significance of the processes that operated in the Troodos magma chamber in elucidating those beneath the axes of oceanic rises has already been discussed[3,4]; here we discuss the genesis and metamorphism of the Sheeted Intrusive Complex and the overlying Pillow Lava Series which, in oceanic terms, are seismic layers 2 and the upper part of 3.

During the past two decades numerous attempts have been made to subdivide realistically this intrusive/extrusive sequence[5-10]. It is reasonable therefore to start with the currently accepted model which is based on primary and secondary petrological differences and on intrusive/extrusive abundance ratios.

Since detailed petrographic and analytical data became available, it has been recognized that there is a primary difference between the two divisions of the Pillow Lava Series (see Fig. 1). Perhaps the most convincing evidence is the abundance of olivine in the upper division and its absence in the oversaturated lavas of the lower group. This, coupled with the varying abundance of dykes, low in the upper and high in the lower division, were the main factors on which the Upper Pillow Lava/Lower Pillow Lava boundary was drawn. But at many localities these various criteria do not coincide nor are they sufficiently obvious to allow an accurate, unique boundary to be drawn. Nevertheless, most workers were in agreement that there were two divisions to the Pillow Lava Series.

Divisions of Pillow Lava Series

Towards the base of the Pillow Lava Series dykes become abundant and commonly form as much as 60% of the outcrop although they maintain the sinuous form common to this division of the massif. Then, within a short lateral distance, they give way to the Sheeted Intrusive Complex with its high dyke density (90%) and planar dyke form. The contact between the two divisions commonly coincides with a marked increase in local topographic relief. The abruptness of this change led some[1,8] to propose that it represents an unconformity, whereas others[3,6,7,9] preferred a gradation, over a short distance, between the two units. At

Table 1 Distinguishing Features on which the Geological Subdivision of the Troodos Massif was Proposed

Sediments	
Upper Pillow Lavas	Generally undersaturated, often olivine-bearing, basalts with more basic varieties (limburgites and picrites) occurring at the top of the sequence. Dykes form less than 10% by volume, absence of silica and celadonite, calcite and analcime common
Boundary	Varying laterally from unconformity to transitional
Lower Pillow Lavas	Mainly oversaturated basalts, often intensely silicified, celadonite common. Dykes, sills and massive flows forming between 30–60% of the outcrop
Boundary	Interpreted as unconformity and gradational
Sheeted Intrusive Complex	Hard, indurated meta-basalts forming an intense dyke swarm of sheeted aspect. Pillow Lava Screens form 10% at most of the outcrop on the upper surface, diminish to zero with depth.
Troodos Plutonic Complex	See refs. 3, 4

the top of the Sheeted Intrusive Complex pillow lavas occur as narrow, elongate screens forming, at most, 10% of the outcrop. With increasing depth they become less abundant until the 100% dyke form of the Sheeted Intrusive Complex is evident.

The distinguishing features on which these divisions were erected are shown in Table 1.

The Troodos structure described above fits well with the seismic data for the oceanic lithosphere. But several workers[11–14] have noted the metamorphic imprint on basaltic rocks dredged from the deep ocean floor. With this in mind, one of us (J. D. S.) began a study of the north side of the Troodos massif where excellent exposures of metabasalts are abundant. We present here the preliminary findings, for they support the seafloor spreading concept, offer an alternative explanation for an oceanic seismic discontinuity and allow the deduction of the metamorphic processes at a constructive margin.

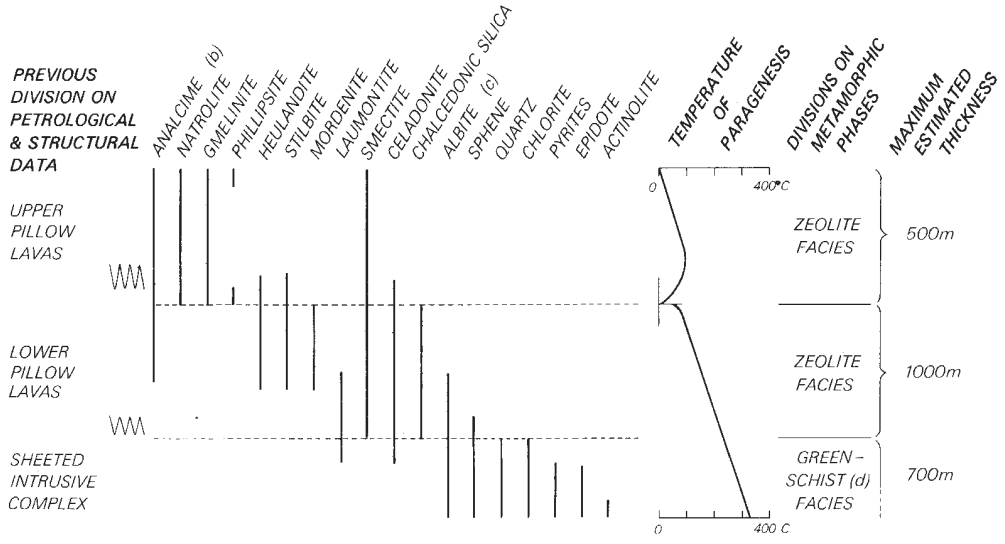
The metamorphic minerals and their distribution within the massif indicate that the previously erected subdivision of the Pillow Lava Series into upper and lower units is valid. As previously suggested[3], however, only the lower division is genetically related to the underlying Sheeted Intrusive Complex. The distribution and temperature parameters of these secondary phases within the three divisions listed in Table 1 are shown in Table 2.

The Upper Pillow Lavas are characterized by the presence of the zeolites, natrolite and gmelinite, which are unique to this division. Phillipsite occurs only at the top and bottom of the sequence and, because it is a zeolite typical of a low temperature (0° C) environment[15], this suggests that these lavas were poured out over a cold oceanic crust and produced their own perched thermal gradient. This proposal is entirely in keeping with the view, based on petrological data, that the Upper Pillow Lavas are genetically distinct from the underlying rocks.

Evidence for Sub-aqueous Erosion

The characteristic zeolite assemblage (see Table 2), together with widespread silicification, allow the Lower Pillow Lavas to be easily identified. This evidence, coupled with the primary morphology and attitude of the lavas, makes it possible to delineate a continuous unconformity between the Upper and Lower Pillow Lavas along the northern flank of the massif. But lavas of the upper division are commonly found directly overlying rocks of the greenschist facies, which supports the proposal that the Upper Pillow Lavas rest unconformably on all underlying formations. A period of extensive sub-aqueous erosion is suggested because: Upper Pillow Lavas rest directly on greenschist facies rocks (see above); neither unmetamorphosed nor submarine weathered basalts occur at the top of the Lower Pillow Lavas which is within a homogeneous zeolite facies; vertical feeder dykes are cut off at the contact; erosional conglomerates are associated with sedimentary sulphide deposits which formed in depressions on the Lower Pillow Lava surface[16]; and unequivocal evidence for widespread submarine erosion occurs on the south side of the massif where extensive sedimentary sequences overlie greenschist facies rocks and are, in turn, overlain by Pillow Lavas (K. Simonian, personal communication).

Table 2 Metamorphic Phases and their Distribution within the Troodos Massif



Temperatures of paragenesis when related to maximum thicknesses of facies suggest a minimum thermal gradient of 150° C km^{-1}. *a*, Chabasite, a rare zeolite on Troodos, has been recorded at two localities in the upper division and one in the lower division of the Pillow Lava Series; *b*, analcime occurs as macroscopic, commonly amygdaloidal, crystals in the upper unit but only in the ground mass of the Lower Pillow Lavas; *c*, the anorthite molecule is metastable within the albite stability field near its upper temperature phase boundary; *d*, we retain the term greenschist but appreciate, as others have done[12–14], that it is inappropriate in an oceanic environment.

Perhaps the most convincing evidence for this strong erosional episode is that it produced such a marked and irregular topography that inliers of the Sheeted Intrusive Complex and Lower Pillow Lavas occur as "islands" completely surrounded by a "sea" of Upper Pillow Lavas.

Metamorphic Boundary

On Troodos, the zeolite facies/greenschist facies boundary is everywhere transitional and nowhere has an intervening prehnite-pumpellyite facies, characteristic of burial metamorphic sequences[17–19], been found. The metamorphic boundary is completely gradational over a vertical distance of between 10 and 20 m. When the vertical exposure is sufficient, any single dyke at the contact shows a transition from a soft, grey–brown rock in the zeolite facies to a hard, light-green–blue rock in the greenschist facies. There is no evidence that this metamorphic contact coincides with any primary structure such as a rapidly changing dyke density; the boundary seems to be entirely independent of the intrusive/extrusive ratio. Thus, in terms of the existing

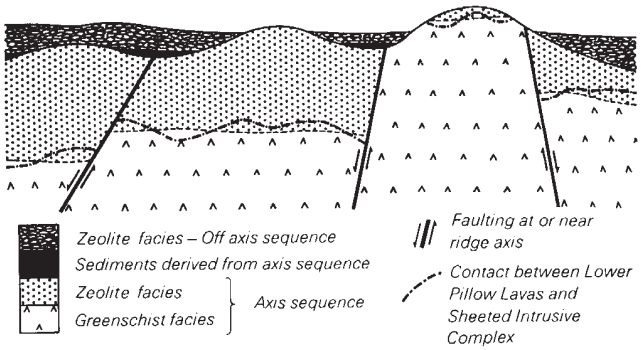


Fig. 2 Schematic cross section showing the relationship of metamorphic facies to units delimited on structural and petrological data.

subdivisions, the metamorphic contact can occur either within the Lower Pillow Lavas or the Sheeted Intrusive Complex. The relation of metamorphic to structural and petrological units is shown diagrammatically in Fig. 2.

Microscopic studies reveal that the metamorphic boundary involves the replacement by quartz and a chlorite mineral of fixed composition (pycnochlorite: $Mg_{2.35}$ $Fe^{2+}{}_{2.6}$ $Al_{1.05}$ $(Si_{2.95}$ $Al_{1.05})$ $O_{10}(OH)_8$ identified from optical data[20]) of the smectite, tentatively identified as nontronite, so common in the zeolite facies above. The incoming of quartz imparts a hardness to the greenschist facies rocks. Below the facies boundary, epidote, and then actinolite, appear as essential phases with increasing depth.

Experimental data[21,22] show that for these metamorphic minerals increasing pressure affects, only slightly, the temperature at which the minerals are stable. Therefore temperature is the prime controlling factor and from the temperature of paragenesis of these minerals estimated predominantly from borehole data in present day active metamorphic regions[23,24], a thermal gradient of 150° C km^{-1} for the Lower Pillow Lavas and Sheeted Intrusive Complex can be deduced. The lowest temperatures of mineral paragenesis and the maximum thicknesses of rock sequences were taken in calculating this thermal gradient. 150° C km^{-1} is therefore a conservative estimate; nevertheless, such a thermal gradient would preclude the existence of a prehnite-pumpellyite facies; this agrees with the field data.

In relating the Troodos evidence to processes at constructive margins, it is evident that only the Sheeted Intrusive Complex and the genetically related Lower Pillow Lavas are, as previously suggested[3], the products of spreading axis processes. The unrelated Upper Pillow Lavas can be best explained as due to off-axis activity, not only after the two lower units had been formed at the constructive margin, but after they had had the metamorphic regime imprinted upon them. Hereafter we refer to the Sheeted Intrusive Complex and the Lower Pillow Lavas collectively as the Axis Sequence and propose that it is a gradational sequence resulting from the injection of dykes and effusion of lavas on or near a ridge axis. As well as fitting with the Troodos data, this proposal is in keeping with experimental evidence[25,26] which indicates that oversaturated basalts such as these could well equilibrate in a high level magma chamber beneath the ridge axis[4,27].

Magmatic and Metamorphic Processes

We follow Cann[11] in envisaging that the axis magma is injected along vertical fissures at or near the ridge axis. We further suggest that the planar structure of the dykes within the Sheeted Intrusive Complex is entirely because the host rock consists, almost entirely, of pre-existing, near-vertical dykes. But when such a dyke enters the homogeneous extrusive pile of overlying pillow lavas it has no structural restraints imposed upon it and finds its way towards the surface along the easiest line of access which is commonly sinuous. This transition from the planar structure in the Sheeted Complex to the overlying pillow lavas with their sinuous dykes was used by previous workers[5–10] to define the boundary between the two units. Although the evidence presented here indicates that the boundary has no petrogenetic significance, we are still at a loss to explain the sometimes extremely rapid upward change in dyke abundance.

Cann's model[11] requires a thermal gradient of some 500° C km^{-1} at ridge axes; indeed, all active oceanic ridges are characterized by high heat flow values. Heat flow profiles across oceanic ridges[27,28] and thermal models derived therefrom[29,30] all suggest that a thermal gradient of 150° C km^{-1} must be well within 100 km of the ridge axis—the wide scatter of heat flow values over the ridges precludes any more accurate predictions. With the Troodos evidence in mind, we suggest metamorphism is imprinted upon the oceanic lithosphere within 100 km of the spreading axis and will take place at a constant distance from the ridge throughout the spreading process. So at this point the metamorphic facies will be produced and the near-horizontal disposition of the greenschist/zeolite facies boundary established as seafloor spreading continues.

We have already noted the variance in physical properties between the rocks of the greenschist and zeolite facies on Troodos. In particular, the presence of quartz in the greenschist facies gives it a rigidity that is distinctly greater than that of the zeolite facies above. This has a marked effect on the seismic velocities, as shown by recent shortline refraction experiments at the Troodos outcrop[31] when velocities averaging 3.2 km s^{-1} were obtained for what are now recognized as the zeolite facies rocks and 4.9 km s^{-1} for those of the greenschist facies. So, although these velocities are low when compared with those from the layers of the oceanic lithosphere, this can be accounted for by the porosity in the near surface rocks of Troodos. Further, it may be significant that the difference in P wave velocities for oceanic layers 2 and 3 is 1.6 km s^{-1}, virtually identical for that between the two metamorphic facies on Troodos. The greenschist/zeolite facies boundary, marking as it does a distinct rigidity difference, could represent the seismic discontinuity between oceanic layers 2 and 3. We are, however, aware that the greenschist/amphibolite facies boundary has been suggested as the metamorphic contact most likely to represent the layer 2-layer 3 seismic discontinuity[32]. The greenschist/amphibolite facies boundary, if present on Troodos must be studied in more detail and velocity of sound in jacketed samples under confining pressure of rocks from the various facies determined, before any more realistic statement can be made concerning the seismic significance of the zeolite/greenschist facies boundary.

The dominant basaltic rocks of the deep ocean basins are quartz and olivine normative tholeiites. So far as we are aware, although alkali basalts have been found[33], more basic lavas such as limburgites and picrites, recorded from the Upper Pillow Lavas of Troodos, have not been dredged from the oceanic rises. We have already presented evidence that the Upper Pillow Lavas of Cyprus are a manifestation of off-axis magmatic activity. The simplest explanation would be that the Upper Pillow Lavas are analogous to the numerous sea-mounts and volcanic islands that embellish the present day oceanic crust away from the ridge axis and are probably produced by thermal plumes rising from the thermally unstable lithosphere/asthenosphere interface. Certainly their undersaturated nature is in keeping with such a proposal as oceanic volcanic island lavas seem to become progressively undersaturated in silica with increasing distance from the ridge axis[28]. But the Upper Pillow Lavas are nowhere more than 500 m thick, show no gross morphological features indicating a localized lava pile, have no extensive underlying

sedimentary unit and related intrusives are structurally concordant with those in the underlying axis sequence rather than having a radial pattern characteristic of oceanic volcanic islands. It seems likely therefore that the Upper Pillow Lavas were erupted on an oceanic rise, relatively close to a spreading axis, before a sedimentary sequence had time to develop and that the products of this activity were nowhere so thick as to allow a high level chamber to develop and produce its characteristic radial fracture pattern.

Whatever the genesis of the off-axis sequence, we propose that, on Troodos, the underlying volcanic rocks represent the products of constructive margin processes, that the rocks were metamorphosed to greenschist and zeolite facies within 100 km of the axis and were subsequently subjected to block faulting and intense local submarine erosion as seafloor spreading continued. The production of this uneven topography near the axis allowed the accumulation of conglomeratic sediments, sedimentary sulphide deposits and later lavas in the bathymetric depressions. Seismic and petrological evidence from the oceanic lithosphere suggests that this model, in part at least, is applicable to the present cycle of seafloor spreading.

We thank J. R. Cann, K. Hsü, D. H. Matthews, E. M. Moores, K. Simonian and F. J. Vine for their help.

Received November 22, 1972.

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A Factor Preventing the Development of Lung Metastases in Rats with Sarcomas

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An immunologically specific factor circulating in lymph and blood prevents the occurrence of lung metastases in rats with sarcomas growing in the leg. Removal of thoracic duct lymph causes such tumours to metastasize to the lung. This is due to the removal of a plasma factor and not of lymphocytes.

THE capacity of rats bearing sarcomas, induced by carcinogenic hydrocarbons, to reject a second subcutaneous challenge with cells from the same tumour is much less than that of rats in which the initial tumour has been surgically removed[1]. The growing tumour has also been shown to prevent the discharge of cytotoxic immunoblasts from the draining node probably as a result of flooding the draining node with tumour-specific antigen[2]. While such tumour-bearing animals show low immunological resistance to tumours at intramuscular sites and impairment of the function of the draining node, yet lung metastases arising from intramuscular tumours are uncommon. We have several established lines of chemically induced sarcomas which when implanted intramuscularly into normal syngeneic rats never metastasize to the lung but which readily give rise to lung tumours following intravenous injections (that is, the cells can grow in the lung but do not do so in tumour-bearing animals). We set out to test the hypothesis that the tumour-bearing rat elaborates a circulating humoral factor which protects against blood-borne tumour cells lodged in the lung and which is relatively ineffective in preventing the growth of intramuscularly inoculated tumour cells.

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Table 1 Effect of Serum and Lymph from Tumour-bearing Rats on Growth in the Lung of Intravenously Injected MC-I Rat Sarcoma Cells

Pretreatment *	10^6 MC-I cells † injected intravenously		2×10^4 MC-I cells † injected intramuscularly	
	No. of rats with lung tumours	Average No. of tumour nodules in lung per rat ‡	No. of animals with tumours	Average weight of tumour (g)
(1) None	10 out of 10	12.1 (2–31)	—	—
(2) Serum from rats with MC-I sarcoma	5 out of 10	2.6 (0–8)	5 out of 5	5.8
(3) Serum from normal rats	9 out of 10	10.1 (0–21)	4 out of 5	7.8
(4) Serum from rats with antigenically unrelated MC-III sarcoma	5 out of 5	8.6 (2–25)	—	—
(5) Lymph plasma from normal rats	10 out of 10	7.9 (1–14)	—	—
(6) Lymph plasma from rats with MC-I sarcoma	6 out of 10	2.7 (0–10)	—	—

According to the Mann-Whitney nonparametric test the difference in the number of lung tumour nodules between group 2 and group 1, 3 or 4 and between groups 2 and 5 is significant at $P < 0.01$.

* Serum (6 ml.) was injected intraperitoneally 4 h before inoculation of tumour cells Thoracic duct lymph (150 ml.) free of cells was injected intraperitoneally in five aliquots of 30 ml. over a period of 24 h, one day before challenge with tumour cells. Serum and lymph were collected from rats that had been inoculated with a mechanically dispersed suspension of tumour 8 days previously and all of which had a growing tumour of between 0.5–1 cm diameter.

† Prepared by trypsin digestion of a solid tumour. Rats were killed 26 days later and tumours weighed.

‡ Values in parentheses are the range of values.

In the first series of experiments the possibility of transferring resistance to tumour challenge with the serum or lymph plasma from tumour-bearing rats was examined and Table 1 shows that, following injection of such serum, rats were resistant to an intravenous challenge (that is, the number of lung tumours was reduced) but the growth of tumour cells injected intramuscularly was unaffected. The tumour used was the MC-I sarcoma which had been induced with methylcholanthrene and is highly antigenic; no intramuscularly growing MC-I tumour has been found to metastasize spontaneously to the lung. That this protection by tumour-bearing serum is immunologically specific is indicated by the failure of serum from rats bearing the immunologically unrelated chemically induced sarcoma, MC-III, to protect against MC-I tumour cells given intravenously. The failure to transfer resistance to an intramuscular challenge passively with serum is, of course, in line with many experiments in the field of transplantation immunity, originating with the classical studies of Billingham *et al.*[3]. Passive transfer of resistance to intravenous challenge has, however, been observed with leukaemias[4].

This experiment encouraged us to explore the possible role of a circulating factor in preventing spontaneous lung metastases arising from sarcomas growing in the legs of rats and which presumably shed tumour cells into the blood. We chose to approach this by cannulating the thoracic duct of rats with established sarcomas growing in the leg, and draining the thoracic duct lymph continuously for six days. After this, the leg with the tumour was amputated; the rats were later killed and the weight of their lung tumours recorded. A number of different chemically induced sarcomas were examined and some, but not all, were found to develop lung metastases following prolonged draining of the thoracic duct. In general, highly antigenic tumours, like the MC-I, could not be induced to metastasize. We studied the mechanism of the induction of lung metastases by removal of thoracic duct lymph with a benzpyrene-induced sarcoma, referred to as "the HSH sarcoma" in its forty-seventh transplant generation. This tumour showed typical individually specific antigenicity and after immunization syngeneic rats were capable of rejecting an intramuscular challenge of 10^5 tumour cells. After removal of thoracic duct lymph from rats with HSH tumours in the leg, lung metastases occurred in every animal compared to an incidence of 20% in rats that had been operated upon but from which lymph had not been drained (Table 2).

The effect of prolonged draining of the thoracic duct is to deplete the pool of circulating lymphocytes so that the

Table 2 Effect of Continuous Removal of Thoracic Duct Lymph from Rats bearing an HSH Sarcoma in the Leg on the Development of Lung Metastases

Treatment	Series 1 (thoracic duct drained in rats with 11-day tumour)		Series 2 (thoracic duct drained in rats with 18-day tumour)	
	No. of rats with lung tumours	Average weight of lung tumours (g)	No. of rats with lung tumours	Average weight of lung tumours (g)
None	0 out of 4	—	0 out of 5	—
"Sham" thoracic duct cannulation *	2 out of 5	0.1	2 out of 4	0.2
Thoracic duct drained	4 out of 4	4.6	5 out of 5	3.0
Thoracic duct drained, lymphocytes returned †	5 out of 5	1.8	4 out of 4	2.7
Thoracic duct drained, serum from rats with HSH tumour given ‡	0 out of 4	—	1 out of 4	0.1
Thoracic duct drained, serum from normal rats given ‡	4 out of 4	2.4	4 out of 4	3.4

The tumour was implanted in leg on day 1, the thoracic duct drained from day 11–16 (series 1) or day 18–24 (series 2). The tumour was amputated immediately after completion of draining thoracic duct and the animal killed on day 61 (series 1) or day 66 (series 2).

* Immediately after cannulation duct was tied off.

† Washed thoracic duct lymphocytes (5×10^8) from tumour-bearing rats were injected intravenously daily during the period of thoracic duct draining.

‡ Serum (4 ml.) from rats bearing an 8-day-old HSH tumour (or from normal rats) was injected intraperitoneally every 12 h during the period of thoracic duct draining.

rats cannot mount a primary immune response[5]. Immunological memory is not abolished, however, and after thoracic duct draining rats retain the capacity to reject in a "second set" manner skin to which they had previously been sensitized[6]. But circulating humoral factors are also removed with the lymph and the levels of immunoglobulins in the blood fall sharply after two days of continuous draining[7]. The experiments summarized in Table 2 indicate that it is the loss of a circulating factor and not of lymphocytes which is responsible for the occurrence of lung metastases following removal of the thoracic duct lymph. The incidence of lung metastases in drained animals was not significantly lowered when washed lymphocytes from the thoracic duct lymph of the same tumour-bearing animals were continuously returned by intravenous injection. That the re-injected lymphocytes rejoined the circulating pool—that is, that they were not so damaged as to be removed from the circulation by the reticulo-endothelial system—is shown (Fig. 1) by the raised

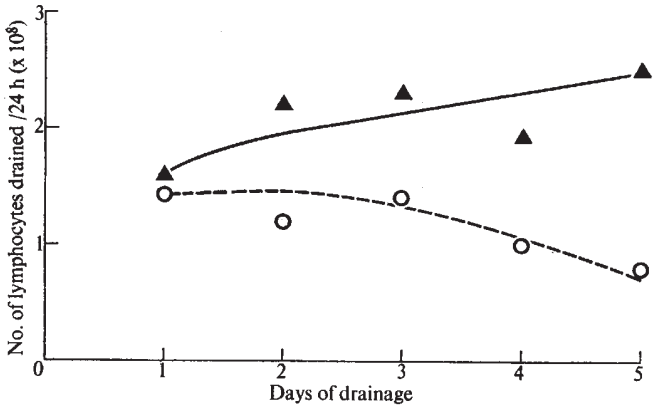

Fig. 1 The effect of returning washed lymphocytes to rats from which thoracic duct lymph has been drained continuously for 5 days. The lymphocytes were collected over 24 h periods at 0° C, washed and returned intravenously (each curve represents the average value from three rats). ▲, Rats with lymphocytes restored; ○, rats with lymphocytes removed. Each point represents the average of three animals.

level of lymphocytes in the lymph of rats which received their lymphocytes back. The direct corollary of this experiment, namely to determine the effect on the incidence of lung metastases of returning the lymph plasma during the period of draining, is technically difficult because of the large volume of lymph removed (that is, up to 150 ml. in each rat per 24 h) and because constituents in the lymph become toxic probably because of autoxidation. Macromolecules present in the thoracic duct lymph accumulate in the blood, however, and consequently another group of rats in the experiments shown in Table 2 received repeated injections of serum collected from a large group of HSH tumour-bearing rats while undergoing removal of thoracic duct lymph over a period of six days. The results clearly show that serum from tumour-bearing animals, but not serum from control rats, prevents the development of lung metastases induced by removal of thoracic duct lymph in spite of the fact that these rats were depleted in circulating lymphocytes.

The active serum factor demonstrated in these experiments is probably produced by circulating lymphocytes, or a cell derived from them, present in tumour-bearing animals because resistance to an intravenous tumour challenge can be transferred with thoracic duct lymphocytes from rats with small tumours (Table 3). As in the case of passive serum transfer, protection was conferred in an immunologically specific way. This experiment is not in conflict with the finding that the return of washed lymphocytes to rats undergoing continuous removal of thoracic duct lymph does not restore the capacity of the rat to prevent the growth of lung metastases. In this last situation, the putative circulating factor even if made by the lymphocytes that have been returned will be lost immediately in the lymph plasma as drainage continues.

Table 3 Effect of Injection of Thoracic Duct Lymphocytes obtained from Rats with a Growing MC-I Tumour on growth in the Lung of Intravenously Injected MC-1 Rat Sarcoma Cells

Pretreatment *	No. of rats with lung tumours	Average No. of tumour nodules in lung per rat †
(1) None	8 out of 8	39.5 (4–58)
(2) Thoracic duct lymphocytes from rats with a growing MC-I tumour	7 out of 8	3 (0–8)
(3) Thoracic duct lymphocytes from rats with antigenically unrelated MC-III tumour	8 out of 8	23.8 (1–58)
(4) Thoracic duct lymphocytes from normal rats	8 out of 8	32.8 (12–51)

* 1.8×10^8 washed thoracic duct lymphocytes were injected 48 h and 24 h before intravenous challenge with 2×10^5 MC-I cells prepared by trypsinization of tumour. The rats were killed 31 days later.

† Figures in parentheses give range of tumour nodules found.

Group 2 differs from groups 1 and 4 with a significance of $P < 0.001$ and from group 3 with $P < 0.005$. Group 3 does not differ significantly from groups 1 and 4.

Presumably, tumour cells are continuously discharged by the tumour into the blood stream and rapidly settle in the lung. The development of these cells into metastatic growths seems to be prevented in the tumours studied in these experiments by a factor which circulates between lymph and blood. This factor exerts its anti-tumour action in the blood stream or the lung but is relatively ineffective at intramuscular sites. Work is in progress to isolate and purify the active principle from serum. It is unlikely to be a conventional antibody (that is, IgG or IgM) directed against the tumour-specific antigens, because in the serum of rats with these sarcomas no free antibody which combines with the cell membrane of the syngeneic sarcoma cells could be detected by either immunofluorescence or mixed cell agglutination[8]. We are exploring the possibility that the material may be related to the specific macrophage arming factor (SMAF)[9], a substance made by T-cells which combines with macrophages and renders these cytotoxic for tumour cells in an immunologically specific way[10]. Such cytotoxic macrophages have been found in the peritoneal cavity of tumour-bearing mice[11].

This investigation was supported by grants from the Medical Research Council and the Cancer Research Campaign.

Received September 29, 1972.

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LETTERS TO NATURE

PHYSICAL SCIENCES

Cometary Collisions and Geological Periods

SOME fifteen years ago, I suggested that tektites were produced by collisions of comets with the Earth[1–3]. Many detailed investigations of these objects have added much to our knowledge, and these, together with the lunar investigations, have proved this hypothesis to be very probably correct. I have also suggested that the geological periods were terminated by such collisions, but this was published in the *Saturday Review of Literature*, and no scientist except me, so far as I know, reads that magazine. The energy of such collisions and their frequency was roughly estimated at that time, and the number of these collisions has been reviewed again by Durrani[4].

The energy of cometary collisions has been considered by several authors (see ref. 5), but to estimate this energy more quantitatively, I consider the energy of a Halley's comet type collision. Cometary orbits which extend to great distances have velocities at the Earth's distance from the Sun of 42.1 km s^{-1}; the Earth's velocity is 29.8 km s^{-1}. If the comet collides head on with the trailing surface of the Earth, the relative velocity is 12.3 km s^{-1}; if with the leading surface it is 71.9 km s^{-1}; and if with intermediate positions and directions the relative velocities are intermediate. Of course, the escape velocity of the Earth, 11.2 km s^{-1}, must be added, and is considerable for trailing type collisions. The two velocities, including this correction, are 16.6 and 72.8 km s^{-1}. The higher velocity corresponds to nineteen times the minimum energy. The higher energy collisions are more probable because comets generally cross the orbit of the Earth. The ones in the larger orbits, at least, move markedly toward and away from the Sun, so the Earth sweeps across their orbits. In the present calculations, I use an effective velocity of collision with the Earth of 45 km s^{-1} though greater or lesser collision velocities are possible.

The masses of comets are largely unknown, but Russell *et al.*[5] and Whipple[6] give reasonable arguments indicating that Halley's comet may have a mass of 2×10^{-9} $M_{\oplus}$ ($\sim10^{18}$) g, and Russell *et al.* suggest that the comet of 1729 may have a mass of 6×10^{21} g. For calculations, I shall use 10^{18} g.

Table 1 gives some estimates based on these assumptions for the effect of a cometary collision with the Earth. The energy, 10^{31} erg, is double the minimum energy required to remove the atmosphere and permit the tektites to be transported to great distances as estimated by Lin[7]. Of course, the energy was not dissipated in only vaporizing water or heating the atmosphere, or heating the ocean and so on, but the data indicate that a very great variation in climatic conditions covering the entire Earth should occur and very violent physical effects should occur over a substantial fraction of the Earth's surface. For example, the great seismic effects might initiate extensive lava flows. The scattering of melted bits of highly siliceous rocks should be only a very small and insignificant part of the physical effects. I suggest that the termination of a geological period would result and a new one would begin.

The scattering of ocean water over land areas would destroy land plants and animals, though probably such water would not fall uniformly and some would not be killed by this method.

Table 1 Energetic Effects a Cometary Collison with Earth Could Produce

Energy to the Earth from Sun in 1 yr	3.48×10^{31} erg
Earthquake of ninth magnitude	2×10^{25} erg
Energy of comet of 10^{18} g and velocity 45 km s^{-1}	10^{31} erg
Fraction of yearly solar energy	0.29
Energy required to remove atmosphere and scatter australites[7]	4.4×10^{30}
If all energy absorbed by	
(1) atmosphere, elevation of temperature	190° C
or (2) ocean water, elevation of temperature	0.175° C
or (3) 100 m of ocean water, elevation of temperature	5° C
or (4) water volatilized at 100° C	4×10^{20} g
Edge of cube to contain this water	74 km
Area of ocean 3 km deep to contain water	1.33×10^{5} km^2
or (5) mass which could be thrown in circle about Earth	3.24×10^{19} g
or (6) earthquakes of ninth magnitude	5×10^{5}

The earthquake effect would be great in the immediate neighbourhood of the collision site, and would be noticeable over the entire Earth. The smog effect due to the ammonia and other compounds of the comet would probably be minor. Because the total energy is equivalent to 0.29 of the energy from the Sun for one year, which would raise the temperature of the atmosphere to 190° C if all heat went into the atmosphere, it seems that a considerable rise in temperature would occur. High temperatures for brief periods would be most destructive to animals and plants, and moderate rises in temperature with high humidity would destroy many living things. It seems that sea animals and plants would fare best if located at some distance where shock would not be important. But would this be true of the air-breathing marine dinosaurs? High humidity and air taken into cool bodies would produce considerable condensation of water in their bodies. Of course, other land based reptiles, such as alligators, as well as the primitive mammals and birds, survived from the Cretaceous into the Palaeocene. Such survival could be due to "good luck"—not all areas were equally affected and some animals and plants took the adverse conditions better than others. But it does seem possible and even probable that a comet collision with the Earth destroyed the dinosaurs and initiated the Tertiary division of geologic time.

Were the ages of Tertiary times determined by the fall of comets which produced the tektite fields? Table 2 lists the ages of these recent geologic periods and the ages of tektites. Rough agreement exists. Errors are probably present in both the geological estimates and the physical measurements of the tektite ages which are my averages of recent measurements. Probable errors in the Moldavites, Libyan Desert Glass and the Bediasites are about 2 m.y. The agreement is satisfactory. I wonder if tektites might not be found at some other boundaries between the Eocene, Palaeocene and Cretaceous periods? Lin[7] required nearly as great an energy as calculated here in order to account for the Indochina and Australian tektites, and this produced only a minor discontinuity in geologic strata, so it seems probable that the energy required for the termination of the Cretaceous was much greater than that estimated here.

Table 2 Ages of Geologic Periods and of Tektites

Geologic period	Ages[8] (m.y.)	Ages[9] (m.y.)	Tektites
Pleistocene		0.71±0.10	Australites
	1	1.2±0.2	Ivory Coast
Pliocene			
	13	14.7±0.7	Moldavites
Miocene			
	25	28.6±2	Libyan Desert Glass
Oligocene			
	36	34.7±2	Bediasites
Eocene			
	58	?	?
Palaeocene			
	63	?	?
Cretaceous			

It seems likely that interesting studies could be made by biologists and palaeontologists in regard to the selection of survivors of such catastrophes. It will most probably be millions of years before the next collision occurs, but survivors of such an event would now most probably need to be able to survive the intense radioactivity from nuclear power plants which will be scattered over the entire Earth's surface. As I stated previously, "If the present suggestion gives the true origin" of tektites and also of breaks in the geologic record, "all will agree that any demonstration of the process would cost far more than the scientific knowledge gained would justify."

I am indebted to Professor Shao-Chi Lin for some suggestions in regard to this paper.

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Received December 1, 1972.

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Origin of Elements

WE believe the recent report in *Nature*[1] under this title to be misleading in the light of recent observations. The recent measurement[2] of $a = {}^{12}C/{}^{13}C = 75\ (+25, -15)$ for the ζ Oph cloud is interesting but not surprising because it can be regarded as a confirmation of earlier work[3], as pointed out in ref. 1, and the conclusion that the relative abundance of ^{13}C in the ζ Oph cloud seems to be terrestrial is quite straightforward. It would be an unwarranted assumption to extrapolate the results from ζ Oph (and other tenuous clouds) to the dense, dusty regions of both the galactic centre and the Orion Nebula.

Zuckerman *et al.*[4] noted the possible presence of regions of high ^{13}C abundance in both Sgr A and Sgr B2 in their initial detection report of the ^{13}C isotope for formaldehyde. Whiteoak and Gardner[5] have continued the study of $H_2{}^{13}CO$ and find optical depths which are consistent with a $^{12}C/^{13}C$ abundance ratio no greater than half the terrestrial ratio—a result which supports an earlier conclusion[6] (from $H_2C^{18}O$ observations) that the $^{12}C/C^{13}$ abundance ratio in Sgr B2 is considerably less than the terrestrial value. In addition, Fomalont and Weliachew[7] have now used interferometric measurements to determine $^{12}C/^{13}C \sim 25 \pm 5$ for Sgr A and ≥ 20 for Sgr B2. We believe that the abundance anomalies in formaldehyde reported by Zuckerman *et al.*[4] have been substantiated by three independent types of subsequent observations.

Within the solar neighbourhood, the HCN detection report[8] ($J = 1$–0) indicated that the $^{12}C/^{13}C$ abundance ratio is possibly anomalous in the Orion Nebula; but saturation effects were unknown at the time. Since then, Wilson *et al.*[9] have measured the $J = 2$–1 transition and reported $H^{13}C^{14}N/H^{12}C^{15}N$ to be consistent with the terrestrial ratio, a result which has been interpreted to mean that the $^{12}C/^{13}C$ abundance ratio is probably normal. Subsequently, the hyperfine components of the J = 1–0 $H^{12}C^{14}N$ line were observed in Orion (L. E. S. and D. B., unpublished) and found to have almost normal intensity ratios—suggesting that this line is not heavily saturated and hence $H^{13}C^{14}N$ may be overabundant. Finally, the recent detection[10] of DCN gives a DCN/HCN abundance ratio more than an order of magnitude greater than terrestrial. Thus abundance ratios determined from measurements of HCN isotopes in the Orion Nebula may well be non-terrestrial; at present the correct interpretation is uncertain.

We note that recent radio measurements[11] of diatomic molecules such as CO give isotopic ratios consistent with terrestrial values in the Orion Nebula. It is possible that simple molecules have abundance ratios close to terrestrial while more complex species do not; thus isotopic abundances may reflect the dominant formation mechanism for each interstellar species. For example, if interstellar CO is formed primarily in the vapour phase, we might expect CO isotopic ratios which are similar to those of the ambient atoms (possibly terrestrial) but, if HCN formation or depletion relies on interstellar dust grains, we may find non-terrestrial HCN isotopic abundances. Optical abundance determinations from diatomic molecules such as CH^+ which are (by necessity) observed in tenuous interstellar clouds should be applied with great caution to dense dusty regions. Finally, although interpretation of radio measurements is often non-trivial, we believe that in the long run radio observations promise to be the most powerful ground-based tool we have for abundance ratios.

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Supersonic Generation of Atmospheric Waves

Chimonas and Hines[1] have pointed out that the Moon's shadow on the Earth's atmosphere during a solar eclipse constitutes a cooling region travelling at supersonic speeds, and may generate atmospheric gravity waves with periods from a couple of minutes up to twelve hours. Atmospheric wave generation by solar eclipses has been observed[2–4], though within the source region (the region of total or partial eclipse) the gravity waves have substantially shorter period than outside it. Here I draw an analogy between the supersonic motion of the Moon's shadow and the supersonic motion of the Earth's terminator. The terminator is supersonic between $\pm 45°$ latitudes at all altitudes below 100 km and may therefore generate gravity waves in this region.

Atmospheric waves are likely to emerge from the regions of solar insolation, namely, the region of molecular oxygen above 90 km altitude, the ozone layer at about 50 km which absorbs solar ultraviolet, the ground, and also possibly from the troposphere where carbon dioxide and water vapour absorb the energy that is reradiated from the ground. It seems unlikely that the tropospheric effect is dominant in the generation of gravity waves because the appropriate high frequency components of the diurnal supersonic motion of the terminator would then be apparent as regular features of ground-based microbarographs. But naturally occurring wavelike fluctuations on ground-based microbarographs are rather rare events that seem to be associated with tropospheric temperature inversions.

The extent of the terminator's source region is far greater than a solar eclipse's source region. At the mesopause the extent of the supersonic motion can be as great as $\pm 55°$ latitude. One would therefore expect atmospheric gravity waves to exist in the ionosphere as a result of the daily supersonic motion of the terminator. This may be the source that is responsible for the almost continuous existence of gravity waves in the ionosphere. If this is indeed the case then a spectral analysis of the gravity waves within the source region should reveal the presence of components of much shorter period than exist outside the source region.

If the trace of the wave's group velocity in the direction parallel to the equator equals the speed of the terminator within the source region an amplification of the atmospheric oscillation should result. If φ is the geographic latitude and θ is the solar declination, which also corresponds to the complement of the angle that the terminator makes with the equator, then to a sufficient approximation in the low and mid-latitudes comprising the source region, amplification of a wave with a horizontal group velocity V_g will occur if

$$V_g = 2\pi R \cos\varphi \cos\theta/(1\ \text{day}) \qquad (1)$$

where R is the radius of the Earth. Because $\varphi < 55°$ and $\theta < 23°$ equation (1) shows that $V_g < 0.9\ C$ where C is the speed of sound. Thus amplification of internal gravity waves cannot occur because, for all internal waves $V_g < 0.9\ C$, the only wave types that can satisfy (1) are evanescent oscillations. The characteristic surface wave $\omega^2 = g\,k_x$ which is the fundamental mode of atmospheric oscillation[5,6], can definitely be amplified. Because the group velocity of the characteristic surface wave is $g/2\omega$, substitution into equation (1) shows that at the equator amplification will occur for a period of 8.9 min at the solstices and 9.6 min at the equinoxes. These periods correspond to horizontal wavelengths of approximately 500 km.

So the terminator may generate a strong evanescent oscillation, corresponding to the fundamental mode of the atmospheric oscillation, in the region within which the terminator's motion is supersonic. In this same region there is liable to be a set of internal gravity waves of longer period than the evanescent waves. It is possible that further interval waves may be generated by a resonant interaction with the waves already extant[7]. Outside the source region a set of internal waves will have been generated in the form of a bow wave. The internal gravity waves outside the region in which the terminator is supersonic will have a longer period than the gravity waves within the source region.

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Received November 28, 1972.

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Magnetic Intensity and Climatic Changes 1925–1970

RELATIONSHIPS between the variations in the Earth's magnetism and climatic changes have been suggested[1–8]. Wollin *et al.*[7] correlated long-period variations in inclination and intensity

Table 1 Geographical Locations of the Magnetic Observatories selected for this Study and the Percentages of Increase or Decrease of Magnetic Intensity (F) within Period Indicated

Observatory	Latitude	Longitude	% Increase and decrease	Period
Abisko	68° 21′ N	18° 49′ E	+1	1929–56
Agincourt	43° 47′ N	79° 16′ W	−5	1900–68
Alibag	18° 38′ N	72° 52′ E	+6	1904–68
Amberly	43° 09′ S	172° 43′ E	< −1	1930–64
Apia	13° 48′ S	171° 46′ W	−1.5	1905–66
Barrow	71° 23′ N	156° 44′ W	< +1	1949–70
Cheltenham	38° 44′ N	76° 50′ W	−5	1901–56
Chelyuskin	77° 43′ N	104° 17′ E	+2	1935–67
Coimbra	40° 13′ N	8° 25′ W	+1.5	1936–68
College	64° 51′ N	147° 43′ W	< +1	1941–70
Dickson	73° 32′ N	80° 33′ W	+1	1933–67
Dombas	62° 04′ N	9° 07′ E	+3	1916–67
Elisabethville	11° 39′ S	27° 28′ E	−1	1932–57
Eskdalemuir	55° 19′ N	3° 12′ W	+1.5	1930–69
Fürstenfeldbruck	48° 09′ N	11° 16′ E	+2	1939–69
Godhavn	69° 14′ N	53° 31′ W	< +1	1945–64
Hartland	50° 59′ N	4° 29′ W	+1.5	1931–69
Helwan	29° 52′ N	30° 20′ E	+6	1908–59
Honolulu	21° 18′ N	158° 06′ W	−6	1902–70
Huancayo	12° 02′ S	75° 20′ W	−5	1922–66
Iasi	47° 11′ N	27° 32′ E	+4	1931–62
Kakioka	36° 13′ N	140° 11′ E	< +1	1929–69
Kuyper	6° 02′ S	106° 44′ E	+1.5	1929–61
Lerwick	60° 08′ N	1° 11′ W	+2	1933–69
Lovö	59° 20′ N	17° 49′ E	+2	1929–67
Meanook	54° 37′ N	113° 20′ W	−2.5	1916–68
Niemegh	52° 04′ N	12° 40′ E	+2	1931–67
Orcadas	60° 44′ S	44° 44′ W	−9	1931–62
Rude Skov	55° 50′ N	12° 27′ E	+2.5	1925–68
San Fernando	36° 27′ N	6° 12′ W	< +1	1928–68
San Juan	18° 28′ N	66° 07′ W	−5	1926–70
Sitka	57° 03′ N	135° 19′ W	−3	1902–70
Sodankyla	67° 22′ N	26° 37′ E	+2	1930–68
Stonyhurst	53° 50′ N	2° 28′ W	+1	1925–66
Tenanarive	18° 55′ S	47° 33′ E	−12	1902–69
Teoloyucan	19° 44′ N	99° 10′ W	−5	1922–68
Tromsø	69° 39′ N	18° 56′ E	+2	1933–69
Valentia	51° 56′ N	10° 15′ W	+1	1936–68
Vassouras	22° 24′ S	43° 39′ W	−4	1919–68
Voyeykovo	59° 57′ N	30° 42′ E	+3	1921–68
Witteveen	52° 48′ N	6° 40′ E	+2	1926–69
Zaymishche	55° 50′ N	48° 51′ E	+3	1920–64
Zuy	52° 27′ N	104° 02′ E	+2.5	1928–58

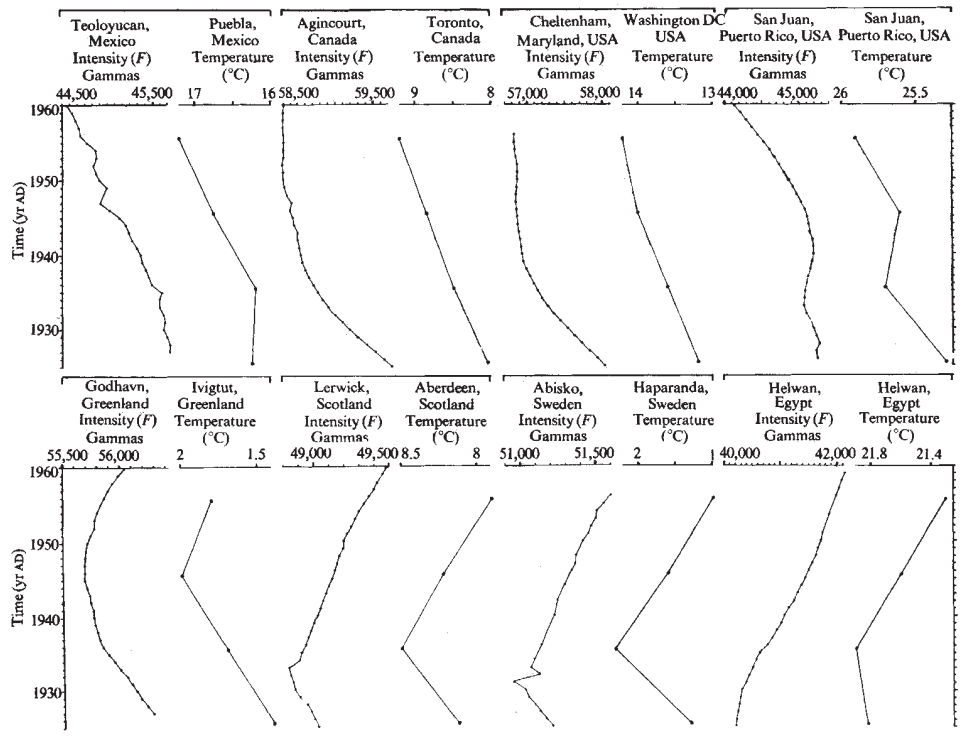

Fig. 1 Annual means of magnetic intensity (F) at individual observatories correlated with the 10-yr means of air temperature from nearby weather stations. Opposite trends in intensity and climate can be observed for different parts of the northern hemisphere.

with evidence of climatic changes from deep-sea sediment cores showing a record of about the past 500,000 yr. In addition, they correlated climatic changes with variations in magnetic intensity based on measurements by Bucha *et al.*[9] in archaeological materials from Arizona, Mexico, Europe, and Asia going back to 8,000 yr ago. Wollin, Ericson, and Ryan[8] extended the correlation between long-period variations of the magnetic intensity and evidence of climatic changes from deep-sea sediment cores back to 1.2 m.y.

We report now the results of correlation between short period changes in magnetism and climate, based on direct instrumental observations. The principal objective of this study was to find out if the recent climatic developments could be correlated to some degree with the changes in the magnetic field.

As a source of magnetic data we used the annual means from more than 200 observatories obtained through the courtesy of the Geomagnetic Data Division, Environmental Data Service, National Oceanic and Atmospheric Administration, US Department of Commerce. As a source of weather data we used the annual and 10-yr means published in World Weather Records[10–13].

We found that the total magnetic intensity (F) has been increasing since about 1930 at most of the observatories in the northern hemisphere and decreasing at most observatories of the southern hemisphere and North America. The magnetic observatories selected for this study are listed in Table 1; they all have intensity records at least 30 yr long or shorter records which show abrupt changes. We then compared the intensity records with weather records on local level. Data from magnetic observatories were always compared with records of the nearest weather station.

Fig. 1 shows total intensity curves based on annual means correlated with 10-yr means of air temperature at the nearest weather stations. The intensity is decreasing at observatories in Mexico, Canada, and the United States while the climate is

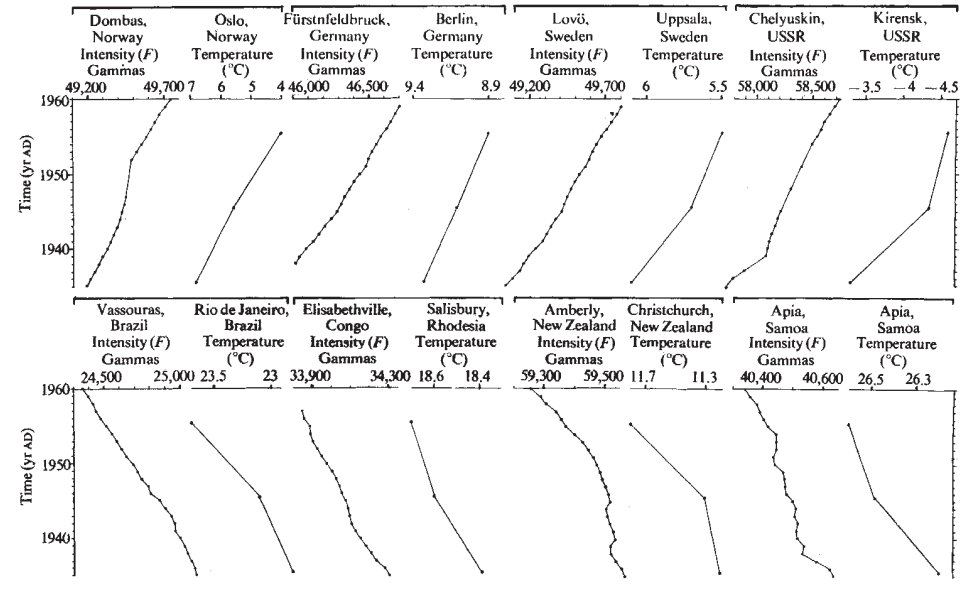

Fig. 2 Annual means of magnetic intensity (F) at individual observatories correlated with 10-yr means of air temperature from nearby weather stations. A comparison of trends in intensity and climate in the northern and southern hemispheres shows that they are opposite for large parts of the two hemispheres.

getting warmer. At observatories in Greenland, Scotland, Sweden, and Egypt the intensity is increasing whereas the climate is getting colder.

In Fig. 2 the trends in magnetic intensity and climate at stations in the northern and southern hemispheres are compared. The trends seem to be opposite for large parts of the two hemispheres. The intensity curves from Norway, Germany, Sweden, and the Soviet Union show increase and the climate is cooling. The intensity in Brazil, South Africa, New Zealand, and Samoa decreases and the temperature is rising. As has been pointed out before, a similar relation was found in central and southern parts of North America.

At several stations (a minority of studied cases) the trends of the 10-yr means of air temperature do not correlate with the trends of the magnetic intensity. At some of these stations, however, the intensity trends correlate with the trends of the winter temperature. In Fig. 3 we show an example of such correlation. The intensity curves from Stonyhurst and Eskdalemuir, Scotland, are correlated with 10-yr running averages of winter air temperatures for central England to 1960 (ref. 14) and for 1960 to 1970 (H. H. Lamb, personal communication).

It may be noted that some places where no positive correlation exists between magnetic intensity and air temperature data are under climatic influence of strong oceanic currents. Examples of such places are Sitka, Alaska, Lima in Peru, Gibraltar, and Tokyo in Japan.

At some observatories there are occasionally abrupt changes from year to year in magnetic intensity. These abrupt changes in magnetic intensity are followed by abrupt changes in weather (Figs. 4 and 5). In general, a lag in temperature trends against magnetic variation by at least 1 yr can be observed.

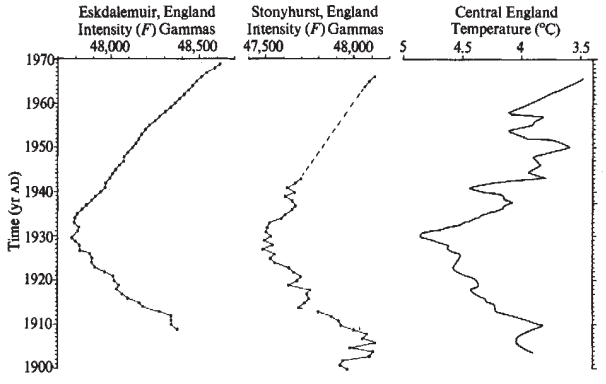

Fig. 3 Magnetic intensity (F) curves based on annual means correlated with 10-yr running averages of winter air temperature for central England to 1960 according to Manley[14] and for 1960 to 1970 according to H. H. Lamb (personal communication).

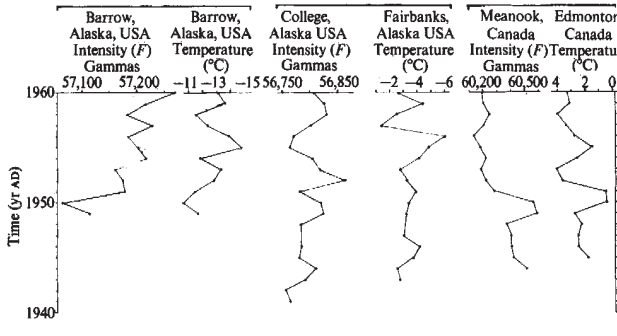

Fig. 4 Correlation of annual means of magnetic intensity (F) from observatories in Alaska and Canada with changes in annual means of air temperature from nearby weather stations. Temperatures lag behind magnetic intensities by one or more years.

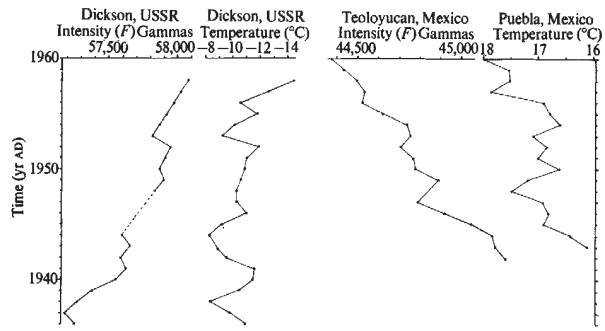

Fig. 5 Correlation of annual means of magnetic intensity (F) from Dickson, USSR, and Teoloyucan, Mexico, with changes in annual means of air temperature trends from nearby weather stations. A 1 yr lag of temperatures behind magnetic intensities can be observed in the Mexican curves.

We have shown correlations between variations in total magnetic intensity and short period climatic changes on a geographical basis. Our tentative conclusion is that the trends in intensity from most of the magnetic observatories in the world with records over at least 30 yr correlate negatively with the 10-yr means of air temperature.

Because of this and other evidence[7,8] we further conclude that a close relationship links changes of the Earth's magnetic field and climate. This may be a direct cause and effect relationship. But Yukutake[15] has suggested that the Earth's magnetic field changes with relation to solar activity and Budyko[16] has shown that the intensity of solar radiation received on ground stations in the northern hemisphere is decreasing since 1938, so we cannot exclude the possibility that both the Earth's magnetic field and climate show parallel reactions to the processes in the Sun.

We thank B. C. Heezen, E. Schreiber, E. Fabiano, K. L. Svendsen, G. E. Stegall, W. T. Hodge, P. D. McLaughlin, R. Drazniowsky, and C. Mayers for assistance. The research was supported by the US National Science Foundation.

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Occurrence of Some Chlorinated Aliphatic Hydrocarbons in the Environment

THE total world production of chlorinated aliphatic hydrocarbons probably exceeds 3×10^6 ton yr^{-1} (ref. 1). Some of the compounds concerned are used chiefly as intermediates in the chemical industry (for example, in the manufacture of polyvinyl chloride, aerosol propellants and refrigerants). But others are employed principally as solvents, particularly for degreasing and dry-cleaning. By contrast with the compounds used as intermediates, for which losses to the environment are unlikely to exceed 1–2%, most of those used as solvents will eventually be lost, chiefly by evaporation but also in effluents. Their loss in the United States alone has been estimated to be $\sim 2 \times 10^5$ ton yr^{-1} (ref. 2). Most of the commercially important chlorinated aliphatic hydrocarbons are comparatively stable compounds, and are likely to have reasonably long lifetimes in the atmosphere and aquatic environment. It is likely therefore that the atmosphere and surface waters would contain significant concentrations of them (particularly those used as solvents). At present there seems to be no information available about the levels of these compounds in the natural reservoirs, nor about the extent to which they are concentrated by aquatic organisms. A project has been initiated recently in these laboratories to examine the environmental distribution of the aliphatic chloro-compounds. We present here some preliminary data for air and natural waters.

We describe the methods used for the analysis of air and water samples briefly; a detailed account is to be published elsewhere[3]. For the analysis of air, a known volume (0.01–0.2 m^3) is aspirated through an activated charcoal trap. On returning to the laboratory the chloro-compounds are desorbed by heating the trap to 250° C in a stream of nitrogen. They are then caught in a cooled trap packed with silicone-coated stationary phase, and are subsequently swept with a current of argon into a gas chromatograph fitted with an electron capture detector. The chlorinated hydrocarbons are stripped from water samples (10–100 ml.) by bubbling with nitrogen[3]. They are then retained in a cooled silicone packed trap and determined by the same technique that was used for gas samples. The estimated coefficient of variation of the method is $< 15\%$ for both air and water samples.

Air sampling has been carried out both in rural areas of Britain, away from large towns (central Exmoor and moorlands of North Wales), and over the North East Atlantic along a line between Cap Blanc (Spanish Sahara) and Lands End. In all instances the principal chlorinated hydrocarbons detected were chloroform, carbon tetrachloride, trichloroethylene and tetrachloroethylene. The concentrations of these compounds varied significantly from one station to another (Table 1). No attempt has been made to interpret the distribution pattern because of the small number of samples available. But it seems that, in general, the concentration ranges of these compounds in the air over the sea are quite similar to those over the land away from urban areas. As would be anticipated, air from towns and cities is considerably enriched with these chlorinated hydrocarbons; thus, air sampled in the precinct of this university on March 25, 1972, was found to contain 18 ng m^{-3} of chloroform, 2.3 ng m^{-3} of carbon tetrachloride, 850 ng m^{-3} of trichloroethylene and 68 ng m^{-3} of tetrachloroethylene. The chromatograms from some air samples contained, in addition to the peaks associated with these compounds, other smaller peaks. Peaks arising from dichlorodifluoromethane trichlorofluoromethane and 1,1,1-trichloroethane have been identified. But a number of others have not yet been characterized.

Analyses have been carried out on samples of surface waters collected from the North East Atlantic in August 1972, during a research cruise aboard RRS Discovery to the area south of the Canary Isles. These showed (Table 2) the presence of significant amounts of the same principal chlorinated hydrocarbons that were detected in the air samples. With the exception of tetrachloroethylene, which had a lower abundance, these were present in ratios which were roughly similar to those in air. As with the air samples, there were considerable variations in the concentrations of these compounds from one station to another. Most samples contained traces of dichlorodifluoromethane, estimated at ~ 2 ng $l.^{-1}$. The chromatograms from

Table 1 Concentrations of Chlorinated Aliphatic Hydrocarbons in Air (ng m^{-3})

Sampling location / Date	Lat.	Long.	Wind direction	$CHCl_3$	CCl_4	$CHCl=CCl_2$	$CCl_2=CCl_2$
Land stations							
	51°10′N	04°20′W	200°	4	0.3	28	17
	53°07′N	04°07′W	220°	6	0.4	15	57
	53°03′N	04°02′W	240°	2	0.7	2	8
	58°05′N	03°50′W	220°	—	0.3	4	—
	53°14′N	03°38′W	220°	2	0.5	4	13
			Average	4	0.4	11	19
Sea stations							
16.7.72	34°19′N	13°32′W	310°	—	0.2	1	3
17.7.72	30°04′N	12°26′W	030°	0.8	0.3	1	8
18.7.72	27°19′N	13°43′W	040°	1.0	0.2	4	2
20.7.72	26°16′N	14°32′W	035°	0.7	0.3	7	2
27.7.72	20°49′N	17°16′W	360°	1.6	0.7	4	7
8.8.72	26°24′N	14°48′W	002°	1.8	0.4	16	3
16.8.72	34°29′N	14°19′W	356°	1.3	0.3	1	1
20.8.72	43°30′N	09°16′W	340°	1.0	0.2	2	5
21.8.72	48°16′N	06°48′W	355°	3.0	0.2	4	1
21.8.72	49°26′N	06°08′W	355°	4.7	0.4	4	9
21.8.72	49°54′N	05°54′W	005°	—	0.6	22	9
			Average	1.7	0.3	6	5

Table 2 Concentrations of some Chlorinated Hydrocarbons in NE Atlantic Surface Water (ng $l.^{-1}$)

Sample No.	Lat.	Long.	Temp. (°C)	Salinity (‰)	$CHCl_3$	CCl_4	$CHCl=CCl_2$	$CCl_2=CCl_2$
A 37	26°14′N	14°53′W	21.0	36.54	4	0.12	7	0.2
A 46	26°09′N	14°46′W	20.4	36.51	8	0.15	10	0.2
A 47	26°07′N	14°50′W	20.9	36.58	8	0.17	—	0.8
B 12	26°21′N	14°50′W	21.5	36.73	13	0.12	5	0.6
B 13	26°10′N	14°56′W	21.3	36.62	9	0.26	8	0.4
B 14	26°15′N	14°38′W	13.6	26.26	8	0.21	11	0.7
Average					8	0.14	7	0.5

several samples contained other small peaks which it has not yet been possible to identify positively.

The data presented here provide information about the general levels of chlorinated aliphatic hydrocarbons in the atmosphere and the sea. Research is continuing to establish the distribution patterns of these compounds in the ocean and other natural waters. A preliminary investigation has shown that molluscs even from the relatively unpolluted waters around Port Erin, Isle of Man, contain in their tissues significant concentrations of several organic chlorine compounds. These include not only those already detected in sea water, but also a number of others (for example, ~0.1 p.p.m. of hexachloroethane). Further work is in progress to investigate the involvement of these compounds in the marine biosphere.

We thank Messrs H. M. Dunlop and P. D. Jones for carrying out sampling at sea. Since this work was carried out, Lovelock *et al.*[5] have reported detection of halogenated hydrocarbons in and over the Atlantic.

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Change in the Current Regime in the Suez Canal after Construction of Aswan High Dam

Exchange of water between the Red Sea and the Mediterranean through the Suez Canal takes place in a seasonal pattern. A northward current dominates the Canal from November to June with maximum velocities during winter. This current is reversed in summer to set in a southward direction with maximum velocities in August–September. But the southward current is generally weaker (in velocity and duration) than the winter northward current. This pattern of circulation was established by comparative study of all available monthly observations of salinity from previous years[1].

One of us (S. A. M., ref. 1) observed an unusual distribution of salinity in the Canal on September 29–30, 1966. Comparison with older data from the Canal in September 1924, September 1933, September 1954 and September 1964 showed that the southward current which had hitherto been observed at that time of the year has been replaced by a weak northward current. Another salinity section was made by El-Sabh on September 17, 1966. The northern part of this section[3], unlike Morcos's section, shows that the northern part of the Canal was filled with Mediterranean water of 38.5–39‰ salinity. This was interpreted[3] as evidence of a southward current. The southern part of the section was published later[4]. Morcos and El-Sabh sections along the southern Canal (between Suez Bay and Great Bitter Lake) show good agreement and indicate a northward flow of the Red Sea water through the Canal.

Strong tidal currents dominate the southern Canal. In winter the northward flood currents are greater in velocity and duration than the southward ebb currents, and as a result the residual non-tidal current is directed northward. In summer opposite conditions occur. The mean sea level at Suez is higher than at the Bitter Lakes in winter, and slightly lower in summer. The currents in the northern Canal depend chiefly on the wind regime and the water balance of the Bitter Lakes, which in turn are a function of the current regime in the southern Canal and evaporation. Evaporation from the Bitter Lakes is an important factor because their surface represents 86% of the total surface of the Canal. In winter part of the water transported northward from the Suez Bay is evaporated in the Lakes and the rest flows to the Mediterranean. In summer, strong evaporation and southward transport to Suez Bay lower the water level in the Lakes, and as a result a feeble current sets northward in the northern Canal aided by the northerly winds and Nile water piling in front of Port Said during the high flood at that time of the year.

The tidal range at Suez is 0.80 and 1.40 m at neap and spring tides respectively compared with only 0.30 m at the northern end of the Canal. The slope of water between the Mediterranean and the Bitter Lakes is much less than between the Lakes and Suez. In the northern Canal, the feeble tidal currents are masked by the non-tidal currents which are much weaker than in the southern Canal, particularly in summer when they become variable in direction with long periods of slack waters (60% of the total time registered by recording current meters in the month of September)[5,6].

The two cruises made in September 1966 show different patterns of salinity distribution in the northern Canal, which may be attributed to the rather slow and variable character of currents in this region. On the other hand, in the southern Canal, where the currents are more stable and dominant, there is a good agreement between the two sections, which confirm the conclusions of ref. 2. Additional support is provided by observations of salinity at Suez during 1966/67 which recently became available[4,7].

Before 1966, the summer southward current in the Canal carried saline water from the Great Bitter Lake to the Suez Bay where higher values of salinity were observed at that season at the southern end of the Canal and even further south in the central part of the Suez Bay. This region is represented in Morcos's observations[1] by station 24 at the most southern buoy of the Canal (162.4 km south of Port Said Lighthouse) and station 25 (164.8 km) at Newport (Zenobia) Lighthouse in

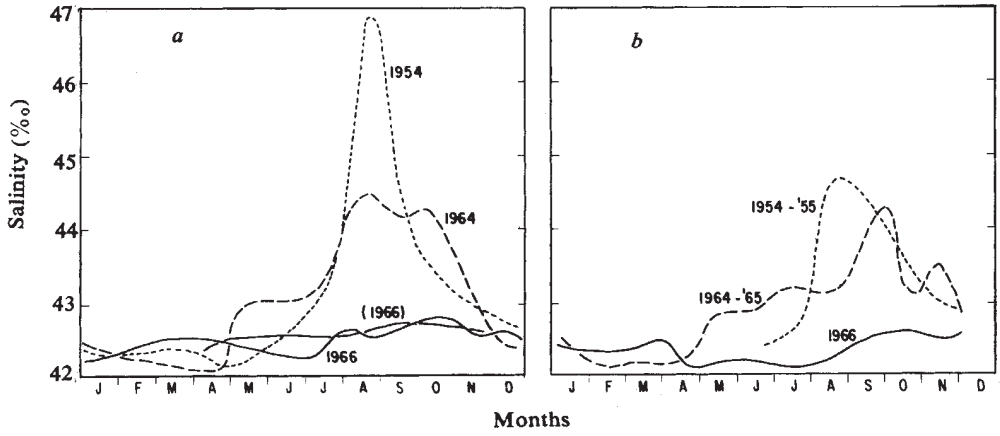

Fig. 1 Seasonal variation of salinity in Suez Bay before and after 1965. *a*, At station 24 (162.4 km, most southern buoy of the Suez Canal). *b*, At station 25 (164.8 km, Newport (Zenobia) Rock Lighthouse). 1954, ref. 1; 1964, our data; 1966, ref. 7; (1966), ref. 4.

the Suez Bay. We possess monthly observations of salinity for these two stations from the years 1924/25 (ref. 8), 1954/55 (ref. 1) and our observations from 1964/65. Further, monthly observations from 1933/34 exist for station 24 (ref. 9). More recently, salinity at these two stations was observed during 17 hydrographic cruises (most of them fortnightly) by Meshal[7] in the Suez Bay from May 3, 1966, to June 5, 1967, and El-Sabh[4] made five monthly salinity observations at station 24 during 1966. Comparison of salinity before and after 1965 at these two stations (Fig. 1) shows a drastic change in the salinity of the Suez Bay. In 1966/67 the salinity remains without exception below 43‰, and much higher values of salinity are observed from July to October during all the preceding sets of observations in both stations. The maximum salinity observed at 12 m depth at station 24 was 42.81‰ in October 1966, compared with 44.40‰ in August 1924, 44.11‰ in September 1933, 46.94‰ in August 1955 and 44.49‰ in August 1964.

Such a change in the salinity of the Suez Bay confirms that the southward current, which has hitherto occurred in the Canal every summer, did not occur in summer 1966, and that the current remained northward during the whole year. It can no longer be argued that the current was reversed in summer 1966 but was either shorter in duration or occurred earlier than usual because the whole summer of 1966 (July to October) is now represented by seven fortnightly observations[7], all of which show that the salinity of the Suez Bay remained below 43‰, and exclude any possibility of a southward current transporting saline water from the Great Bitter Lake.

In summer 1966, and for the first time, the huge amounts of Nile water which have hitherto flowed in to the Mediterranean were completely prevented by the Aswan High Dam which was finished before the flood of 1966. Whether this change in the current regime is entirely linked to the Aswan High Dam, and whether this change continued to occur every year after the summer of 1966, are questions open to future investigations.

If this phenomenon is permanent biological migration between the Red Sea and the Mediterranean will be promoted. From recent observations of the distribution and ecology of *Ceratium egyptiacum* Halim, Dowidar[10] gave biological evidence confirming the new regime of currents in the Suez Canal.

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Ramgarh Structure, India

CRAWFORD[1], in his letter concerning the Ramgarh structure, a possible impact crater in India, seems not to be aware that the area has now been geologically mapped in detail. During geomorphological mapping of the Lower Chambal Valley in 1968, I encountered this feature and mapped it[2]. My chief findings are that: the Ramgarh dome is, topographically, a circular basin and structurally a dome having quaquaversal dip direction.

This sedimentary dome of typical shape is especially interesting because it displays an inversion of topography. From the Devi temple on the eastern escarpment this basin is seen to be

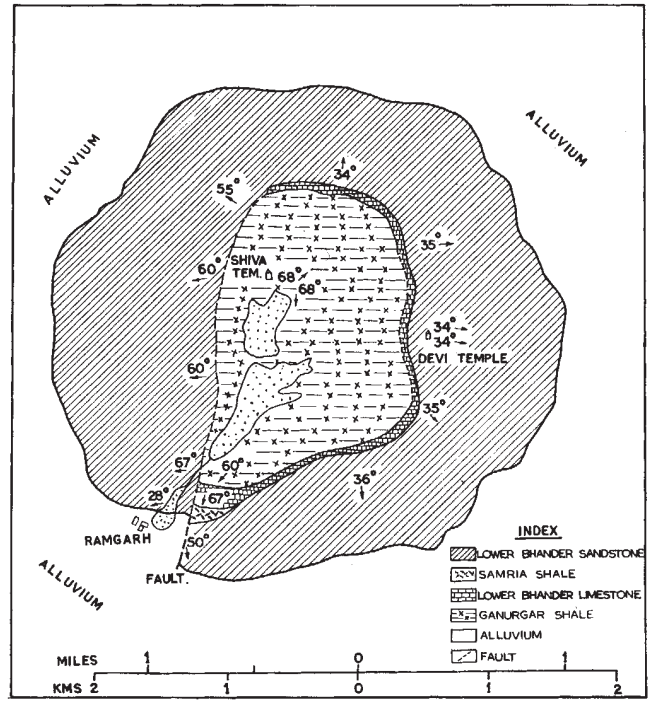

Fig. 1 Geology of Ramgarh dome.

bounded by a precipitating circular wall of Lower Bhander Sandstone nearly 150 m high, underlain by Lower Bhander Limestone and Genurgarh shales which form the lower half of the scarp. The shales are displayed in the form of a circular depression within a dome (Fig. 1).

The amount of dip of Lower Bhander Sandstone ranges from 32° to 70° but mostly close to 35°. Along the western side, the fault contact running north–south brings the Ganurgarh shales and Lower Bhander Limestone in a direct contact with Lower Bhander Sandstones. Because of faulting, the inclination of beds here is unusually high, from 60° to nearly vertical.

The faulting in the dome is indicated by the sudden steep dip of sandstone, truncation of rocks of varying resistance, intense fracturing and close jointing of sandstones and shales, and shattering and brecciation in the sandstones.

Minor folds are also evident in the rocks and are responsible for the local thickening of beds. Within the core of the dome to the east of the Shiva temple, in a streamcut channel, the Ganurgarh shales intercalated with limestone have been exposed. Here the beds plunge towards NNE and SSW at steep angles (about 67°).

Regarding the origin of this dome it seems on the basis of structural and geomorphic features that such a crater-like feature could not have been produced without any support from beneath. Further the Ramgarh dome seems to be a combined result of tectonic and volcanic activity. Thus, the suggestion that this feature is a result of tectonic movement of diapiric nature does not seem convincing.

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Ramgarh Structure, India

CRAWFORD[1] has discussed the possible impact origin of the Ramgarh structure in Rajasthan State, India. He mentioned the find of a shatter coned specimen in the colluvium near the centre of the structure. On the other hand, Auden[2] has commented that the structure may be a kimberlite type of intrusion into the Upper Vindhyans similar to that at Majhgawan in Madhya Pradesh, India. No detailed work has so far been attempted to prove the origin of the structure. The area was visited by Mallet[3] and first mapped on a small scale (1 : 63,360) by Kishen Singh (unpublished). But neither give any conclusive evidence to explain the origin of the anomalous structure.

One of us (A. D.) made an initial visit to the Ramgarh area in July 1972, to find evidence of impact, if any, on the surface in and around the Ramgarh structure. A similar study of the Lonar crater in Buldana district, Maharashtra, India, proved conclusively that that crater is of impact origin (K. Fredriksson *et al.*, in preparation). Samples of rocks were collected from different parts of the Ramgarh structure. Examination of the structure in the field showed abundant evidence of shear fracturing in otherwise massive quartzite. Preliminary microscopic examination of the quartzite has revealed that the quartz grains along closely spaced fractures are granulated and show anomalous birefringence. The evidence, though inconclusive, supports the theory of the formation of the structure by impact.

A detailed investigation of the structure by drilling, pitting and trenching to determine the origin of this controversial structure has been planned by the Geological Survey of India.

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Recent Serpulid Reefs, Connemara, Eire

A SHELTERED lagoon, Ardbear Lough, Clifden, Co. Galway (Irish National Grid L 66 49), provides a very favourable environment for the growth of fringing reefs of the calcareous tubed polychaete *Serpula vermicularis* L. As far as I am aware living serpulid reefs (as distinct from encrustations) have not been described previously. Fossil reefs are described from the Lower Carboniferous of Cumberland (ref. 1 and M. R. Leeder, in this issue) and sub-fossil reefs from Baffin Bay, Texas[2]. The occurrence of a living serpulid reef is of geological and biological importance and here I describe in outline the ecology of the reefs.

Serpulid reefs occupy about one-third of the area of the lagoon (total area of lagoon about 1 km^2), chiefly around the perimeter and islands where a rocky substrate is exposed. The lagoon bottom is mud. Shell gravels occur locally in areas of high carbonate production at the lagoon mouth. Terrigenous gravels are found on shores where waves erode glacial deposits.

The biocoenosis is first formed by encrusting and then upward free growth from larvae settling on rock, boulder and gravel sized clasts. Tubes are built so that their apertures face upwards and outwards to a length of up to 20 cm so that the

Fig. 1 Detail of serpulid colony illustrating tubes of *Serpula vermicularis* (arrows point to trumpet like structures) encrusted with *Pomatoceras triqueter*, bryozoans and spirorbids.

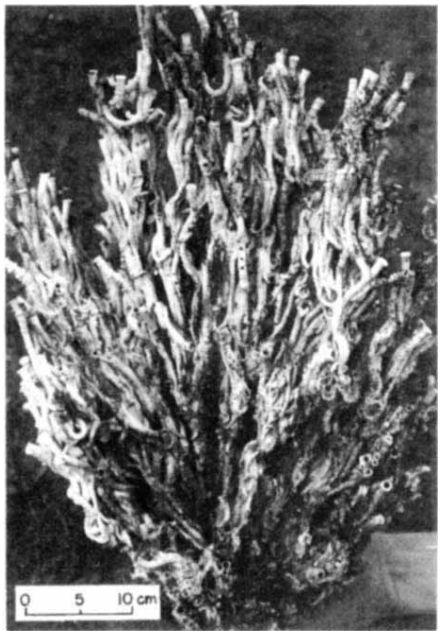

Fig. 2 Section of reef showing growth structures.

worms are positioned most favourably for filter feeding. Increase in colony size follows by larval settlement on old tubes (Fig. 1). Growth of tubes measured over one month (August 1972) averaged 9 mm. Growth is periodic as shown by trumpet-like structures on tubes (Figs. 1 and 2). The older parts of the colony are eroded by algae and sponges, and become very fragile as they age. Segments of the reef frequently break off and lie on the bottom where they form large new areas for larval settlement. This is the principal way in which the reef structure is built upwards and outwards. The greatest reef development occurs at depths between 3 and 19 m where individual colonies may be as much as 2 m high.

The reef provides an attractive site for a varied epifauna which includes the following carbonate secreting organisms: bryozoans, spirorbids, bivalves (byssate sessile) and epifloral calcareous algae. Predators include *Labrus melops* which bites tubes open to feed on the worms and *Asterias rubens* which inverts its stomach down the tube to ingest worms (comparable to the parrot fish and *Ancanthaster planci* of tropical coral reefs).

Currents are not strong enough to transport reef debris or to bring in allochthonous fine sand or larger sized clasts so that an *in situ* carbonate deposit is being formed with a muddy matrix.

I thank Drs R. Goldring, G. Warner, R. Till, D. Helm and M. Leeder for criticism and Mr C. Gray for assistance with diving.

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Lower Carboniferous Serpulid Patch Reefs, Bioherms and Biostromes

AUTOCHTHONOUS patch reefs, bioherms and biostromes composed of calcareous-tubed serpulids occur in the Lower Carboniferous (Tournaisian) Lower Border Group of Cumberland and Roxburghshire. Serpulid colonies on this scale were previously unknown in the geological record. Living reefs, from a lagoon in Co. Galway, Eire, have only recently been discovered[1] although dead reefs are common in Baffin Bay, Texas[2,3]. Some of the Carboniferous serpulid horizons were originally discovered by Garwood[4] who briefly referred to them as "worm beds" and assigned the tubes to *Serpula cf. advena* Salter. All the serpulid tubes are calcitic, show concentric microstructure identical to that of recent serpulids, and have small internal diameters (0.5–3.5 mm); an unusual feature is thin septae dividing the tubes (Fig. 3), structures unknown in recent serpulids and which necessitate a complete taxonomic revision.

Thin biostromes and bioherms (Fig. 1*b*, *c*) are common in the carbonate members of the Lynebank and Liddel Formations where they may be associated with stromatolites. The tubes within these growth forms are loosely packed and show gently sinuous upward growth. Accretion of the lens-shaped bioherm (Fig. 1*b*) is thought to have kept pace with quiet water clastic and carbonate mud sedimentation in surrounding areas. The depression at the top of the biostrome (Fig. 1*c*) is filled with bioclastic debris and indicates a minimum relief of 30 cm during the last stages of growth.

The best developed patch reef is exposed in Stack Cleugh near Bewcastle, where it forms part of the basal limestone of the Main Algal Formation (Fig. 1*a*). The patch reef, 13 m long and over 2 m high, tongues out to the west into 2 m of thinly bedded calcitic micrites of quiet water origin. To the east the reef reaches a maximum thickness of 2.3 m and ends

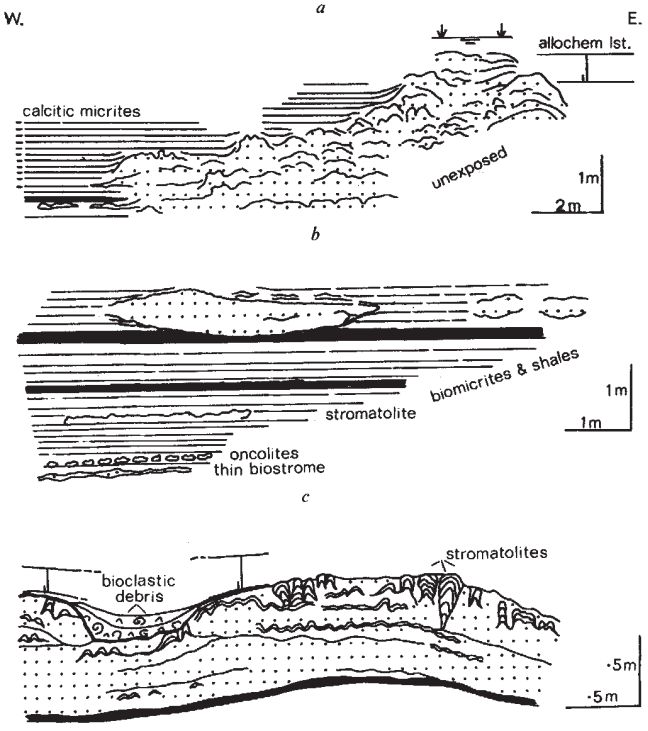

Fig. 1 Field sketches of serpulid colonies. *a*, Patch reef from Stack Cleugh, Bewcastle (NY 58887464), showing internal relief features. *b*, Lens-shaped bioherms from River White Lyne, Bewcastle (NY 54547604), Lynebank Formation. *c*, Part of serpulid biostrome exposed at top of Larriston Quarry, Newcastleton (NY 55739380), Liddel Formation, showing intergrowth of stromatolites in upper part.

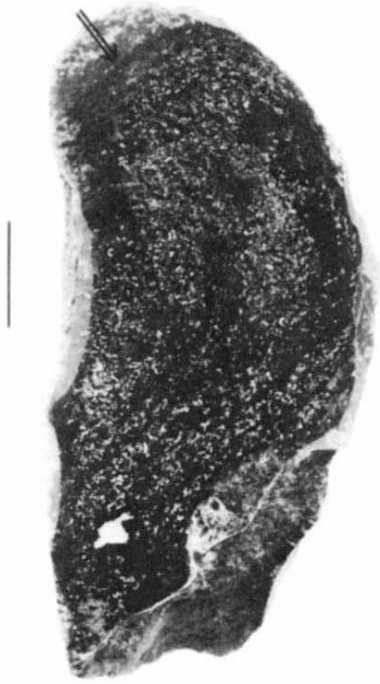

Fig. 2 Polished and etched vertical section through ridge growth form from the patch reef shown in Fig. 1*a*. Thin, closely packed tubes and periodic halts in ridge accretion indicated by dark micritic laminae (arrowed). Scale bar = 2 cm.

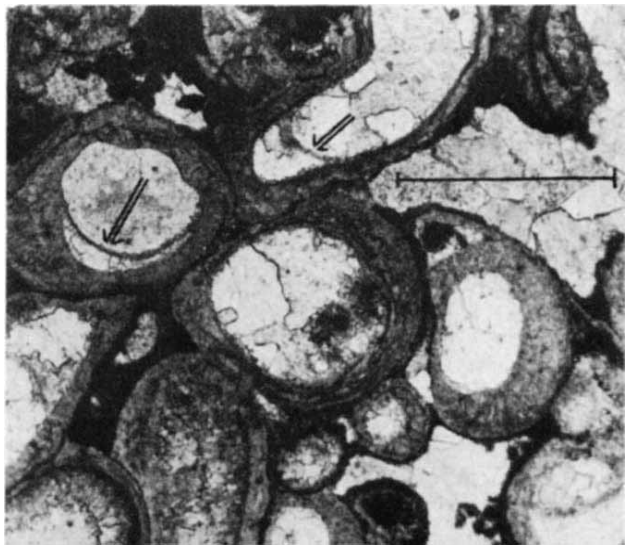

Fig. 3 Photomicrograph to show closely packed, cemented serpulid tubes from ridge growth form of Fig. 2. Septae within oblique sections (arrowed). Tubes infilled with ferroan calcite cement and set in micritic matrix (appears dark) which is partly recrystallized. Scale bar = 1 mm.

abruptly along a steeply dipping surface interpreted as original reef topography. Internally the patch reef is composed of mound, ridge (Fig. 2) and dome growth forms up to 30 cm high and 50 cm amplitude. Periodic growth halts are recorded by encrusting micrite laminae of calcareous algal origin (Fig. 2). Individual tubes are tightly packed and serial sections show irregular, sinuous coiling parallel to ridge and dome growth surfaces. Tubes are cemented to adjacent individuals (Fig. 3) and are surrounded by partly recrystallized calcitic micrite matrix containing calcareous algae. Absence of tube debris within the patch reef and in adjacent sediments indicates that the reef formed a compact, resistate structure during life.

Sedimentological work in progress and ecological studies of recent reefs may help explain the significance, dynamics and exceptional development of these fossil serpulid colonies.

I thank Drs R. Goldring, R. Till and Mr D. W. J. Bosence for discussions and helpful criticisms, and Reading University for a research studentship.

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Received November 1, 1972.

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Recent Vertical Crustal Movements between the Dead Sea Rift and the Mediterranean

Analysis of repeated precise surveying of the geodetic network in Israel, presented below, reveals recent crustal movements. The region studied consists mainly of folded sediments disrupted by faulting, which increases in intensity from the Negev in the south towards Galilee in the north (Figs. 1*A* and 3*A*). On the east the structure and morphology are determined by the Dead Sea–Jordan Rift (a segment of the East African Rift system) and its subsidiary fractures, tilted blocks and volcanism[1–3]. On the west, a major fault is inferred along the Mediterranean shelf (ref. 4 and Neev and Bakler, personal communication). Much of the tectonic activity is post-Tertiary, but in spite of numerous traces of young crustal activity[5–13], the seismic record indicates a quiet regime with only sporadic stronger tremors[14].

Our study is based on the 1962 and 1969 levellings of the geodetic network in Galilee, and on the 1959 and 1966 levellings of two long traverses across the Negev. The operations conformed to specifications of first-order precise levelling[15,16], the discrepancy (E) between the forward and backward measurement of each segment not exceeding ($3\sqrt{D}$) mm (D, length of a segment in km). Equipment, precision of measurement, and monumentation are of the kind used in study of recent crustal movements in other regions[17–19].

To be significant in a study of recent crustal activity, the differences (dH) between positions of individual benchmarks, determined in two successive surveys, must exceed the range of geodetic errors and must be independent of them. The dH values computed by us range from -65 mm to $+55$ mm (for the 7 yr span) and exceed considerably not only the range of errors based on the adjusted mean square values (0.3–0.6 mm/km), but also the range of errors based on the actual E values (Fig. 2). The plots of both the algebraic and absolute values of dH against those of E, and against D, yield diffuse patterns, a result confirmed also by Kendall rank correlation tests. We assume, therefore, that the dH values obtained in this study are significant and reflect crustal activity. It should be stressed, however, that deep knowledge of all sources of systematic and accidental errors in levelling is still lacking[15].

In regions such as the Russian Platform and Fennoscandia the crust is assumed to rise and sink *en bloc*, and regional rates of movement are often quoted as averages[17–19]. In our case the number of positive and negative dH values is about equal, and averaging them would result in a false picture of apparent stability. However, when the dH are plotted in geological cross-sections and maps, a definite pattern of movement

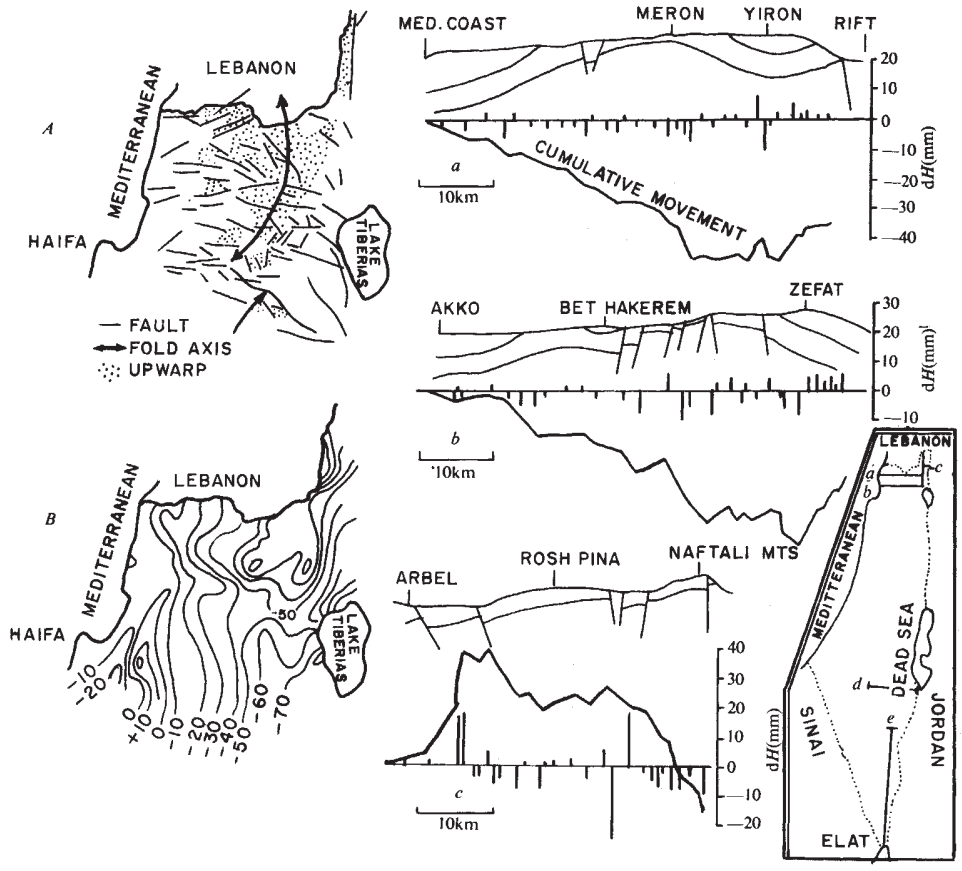

Fig. 1 *A*, Faulting in Galilee; *B*, contour map. *a*, *b* and *c*, Three traverses as shown in inset.

emerges. Fig. 1 shows the distribution of dH values in the intensely faulted Galilee. This mountainous region is uparched along a roughly N–S trending axis, but the folding is obscured by three sets of faults, oriented E–W, NE–SW, and NW–SE, which produce complex structures such as tilted blocks in Eastern and Western Galilee, and horsts and grabens in Central Galilee[1–3]. The cross sections indicate a clustering of positive dH values along structural lows, and of negative values along the highs. The numerous reversals in sign of dH values across faults support the idea that some faults in this region are still active[1]. The relatively sparse spacing of benchmarks does not allow analysis of movement of the individual structures, but the regional pattern is revealed by the contour map of Fig. 1*B*. The contours represent the cumulative dH, computed relative to Benchmark F/30 near Akko on the Mediterranean coast; they are therefore contours of displacement relative to an arbitrarily chosen point. The map shows relative sinking of the structural backbone of Galilee with respect to the Rift margins and the coastal zone. It appears also that the dH values are somewhat higher along the Jordan Valley segment of the Rift, and along edges of the Yizre'el Valley, where the seismic activity also appears to be higher than in the surrounding areas[14]. No levelling has yet been conducted across the Rift, and the possible movement of its floor cannot be assessed. The N–S cross-section (along the western edge of the Rift), shown in Fig. 1, demonstrates, however, a complex pattern which may be related to the fragmentation of the Rift floor or/and to the subsidiary fractures of the Rift.

Similar relationships were determined in the two traverses across the Negev[20], which is dominated by a series of NE–SW aligned folds locally disrupted by faulting, and transected on the east by the Rift (Fig. 3*A*). Geological evidence suggests young Quaternary subsidence of the Rift interior, accompanied by some differential movements and a rise of the Rift edges[5,7–9,12], some recent faulting, and subsidence of some shallow sabkha zones[12]. The cross-sections in Fig. 3 show the vertical displacements along the levelling paths and confirm the morphotectonic control of their distribution along faulted as well as folded zones[20].

The above evidence suggests that during the past decade differential vertical crustal movements, up to several centimetres in magnitude, have occurred along the rift borders, The inverse relationship between the morphotectonic features and the dH distribution, that is, the subsidence of structural highs and the elevation of lows, is striking and suggests gravity compensation effects. Because, however, the morphotectonic features shown in Figs. 1 and 3 show a close agreement between structure and topography, the question arises whether the dH distribution reflects the true displacement pattern or whether it is caused by some unknown operational error related to topography or gravity. Theoretical considerations[21] and step-by-step examinations of the precise levelling techniques and operational procedures have failed to reveal any possible source for such a systematically misleading relationship between the topography and dH values.

The magnitude of possible recent crustal activity indicated in this study is similar to that reported from other regions[17–19],

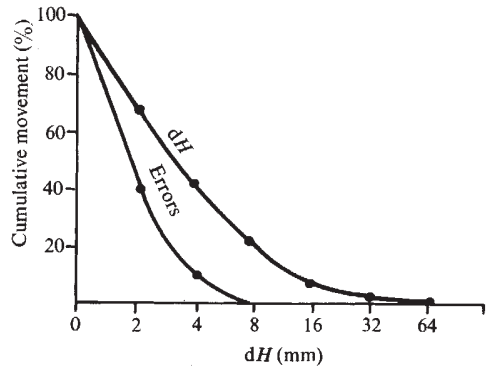

Fig. 2

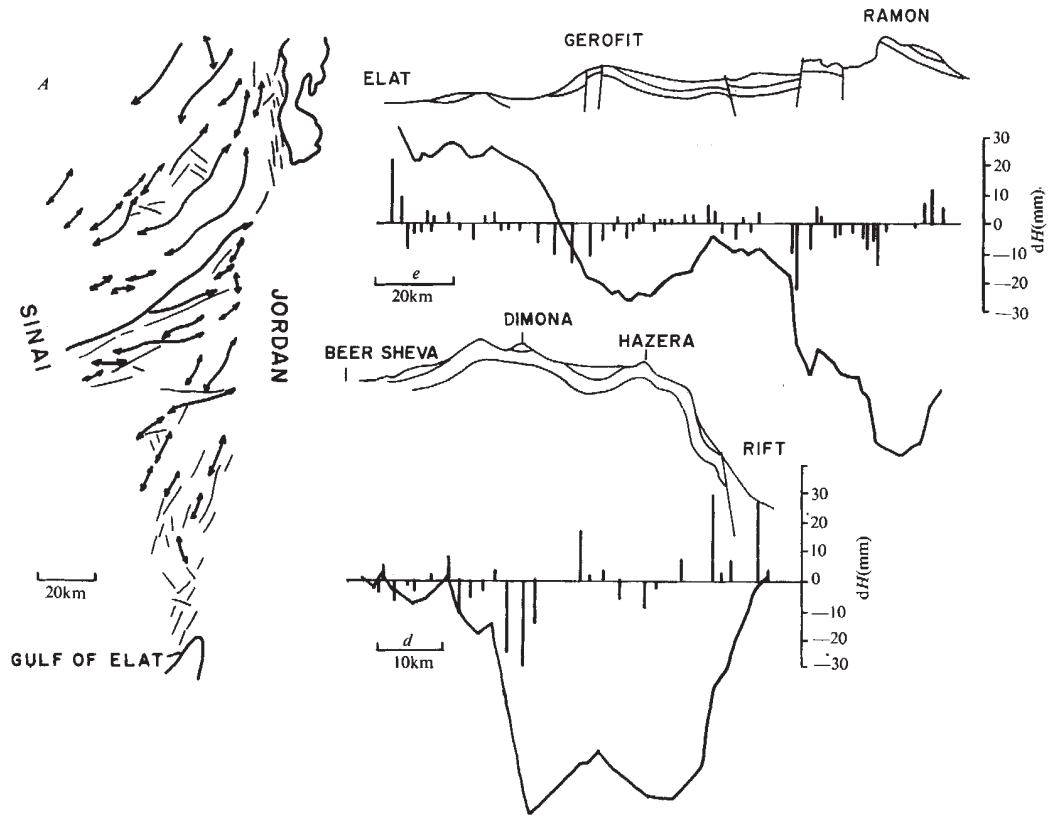

Fig. 3 *A*, The Negev faulting. *d* and *e*, Two long traverses across the Negev, shown in Fig. 1 inset.

but exceeds the long-term rates of tectonic activity, usually quoted in geological literature. Only future levellings will demonstrate whether the movements inferred here persist in signal and magnitude, or whether they oscillate in time and space.

We thank J. Elster, L. Fish, B. Schmutter, U. Shoshani, and D. Karmeli, for help and advice, and help from the US National Academy of Sciences is acknowledged.

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Received April 18; revised September 29, 1972.

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BIOLOGICAL SCIENCES

Homology between Epstein-Barr Virus DNA and Viral DNA from Burkitt's Lymphoma and Nasopharyngeal Carcinoma determined by DNA-DNA Reassociation Kinetics

THE detection of Epstein-Barr virus (EBV) DNA in established lines of lymphocytes and in biopsies of Burkitt's lymphoma and nasopharyngeal carcinoma has been reported[1-4]. The average number of EBV genomes associated with virus nonproductive cells was estimated as forty to one hundred per cell by a nucleic acid hybridization technique with EBV-specific complementary RNA (cRNA) and cellular DNA[3,4], whereas two to five genomes per cell were found by DNA–DNA hybridization on nitrocellulose filters[1,2].

The number of genome equivalents in Simian Virus 40 (SV40) transformed cells determined by the cRNA hybridization method gave values overestimated ten-fold[5] as compared to DNA–DNA reassociation kinetics studies[6]. The number of EBV genomes in virus nonproductive cells found with the cRNA method was relatively high (0.06–0.2% of the total cell DNA), and might also have been an overestimation due to technique. We therefore conducted DNA–DNA reassociation kinetics studies to re-estimate the number of genomes in nonproductive cells (Raji). This technique also shows whether the virus DNA in test cells or biopsies is identical to the EBV DNA isolated from HR1K (EBV-productive) cells.

Two of the three methods available to produce highly radioactive EBV DNA were unsuitable. It was too difficult to handle the large volume of radioactive culture produced by simple addition of ^{3}H-thymidine and the resultant specific activity was only about 10^4 c.p.m. μg^{-1}; there was no guarantee of uniform transcription of whole EBV DNA by use of reverse transcriptase *in vitro* with ^{3}H-TTP. The third makes

use of repair-type replication with DNA polymerase I (Kornberg enzyme)[7] and it is reasonable to expect EBV DNA would be evenly repaired at low temperature and therefore evenly labelled with ^{3}H-TTP if nicks are first introduced by small amounts of DNAase I. A low temperature of 15–20° C is required to restrict the reaction to repair-type synthesis without extensive displacement replication of DNA[8]. In this case the reaction initiates with removal of nucleotides from nicked points by activation of 5′ to 3′ exonuclease, and polymerization primed on the 3′ ends of the nicked points follows[7].

EBV DNA was extracted from partially purified virus particles and purified by sucrose gradient centrifugation and two cycles of density equilibrium centrifugation in CsCl as described previously[4].

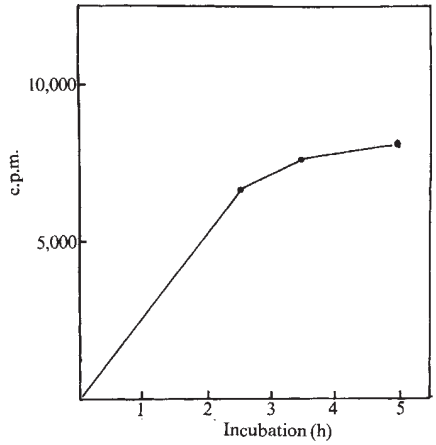

Fig. 1 Time course of ^{3}H-TTP incorporation into EBV DNA by repair synthesis. Two μg of purified EBV DNA was incubated with 0.1 μg of DNAase I at 37° C for 15 min in 1 ml. of the polymerase buffer (potassium phosphate 70 mM, *p*H 7.4, $MgCl_2$ 7 mM, and 2-mercaptoethanol 1 mM); the enzyme was inactivated by heating at 70° C for 10 min. This DNA solution was diluted with the reaction mixture so that 1 ml. of the reaction mixture should contain 70 μm of potassium phosphate buffer, *p*H 7.4, 7.0 μm of $MgCl_2$, 1 μm of 2-mercaptoethanol, 0.1 μm each of dCTP, dGTP, dATP, 0.3 μm of ^{3}H-TTP (58 c mmol^{-1}), 1 unit of enzyme and 1 μg of EBV DNA. This was incubated at 17° C, and aliquots were taken to measure the incorporation of ^{3}H-TTP into TCA-precipitable materials at 2.5, 3.5 and 5 h.

Fig. 1 shows the kinetics of ^{3}H-TTP incorporation. The reaction approached a plateau in 3 h followed by a slight further increase. It was terminated by addition of 'Sarkosyl 97' to a final concentration of 1% and heating at 70° C for 5 min. Specific activity, determined by measurement of the radioactivity of TCA precipitates of a portion of the reaction mixture at termination (5 h), was 5.25×10^6 c.p.m. μg^{-1} of EBV DNA.

Figs. 2*a* and 2*b* show the sedimentation characteristics of template (original) and product (repaired) EBV DNA in native forms, both with a similar distribution with a 10S peak that corresponds to 8×10^5 daltons[10]. Figs. 2*c* and 2*d* are sedimentation profiles of the denatured product and template both with a 6 to 7S peak, which corresponds to 10^5 daltons[11], with some trailing toward the higher molecular weight region. There was therefore an average of 6 nicks per native 10S DNA strand. When ^{14}C labelled DNA from HeLa cells was nicked and repaired with ^{3}H-TTP as described above, the product and the template DNA had the same sedimentation profiles. Incubation with the polymerase therefore did not change the size of DNA.

We estimated the number of EBV genomes in Raji cells, a virus-nonproducing cell, using the highly labelled EBV DNA. DNA was extracted from cells by treatment with pronase, 1 mg ml.$^{-1}$ and SDS 1% in Tris buffer 0.05 M, *p*H 9.0, at

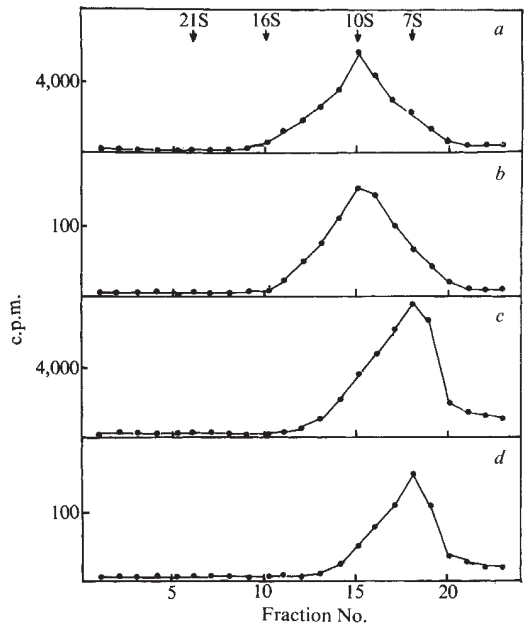

Fig. 2 Sedimentation of EBV DNA product and template in native and denatured form. EBV DNA was originally labelled with ^{3}H-thymidine with a specific activity of 2×10^3 c.p.m. μg^{-1}. This DNA was nicked, reacted with DNA polymerase I in the presence of ^{3}H-TTP (58 Ci mmol^{-1}), and purified. The mixture was passed through 'Sephadex G-25' equilibrated with Tris buffer 0.01 M, EDTA 0.001 M, and 'Sarkosyl 97' 0.1%. The DNA fractions were treated with water-saturated phenol and dialysed against SSC (NaCl 0.15 M, sodium citrate 0.015 M). The most purified fraction (IX) of DNA polymerase I was prepared according to Richardson *et al.*[9] and had a specific activity of 13,000 units mg^{-1} of protein determined with dAT polymer as a primer. Centrifugation was conducted at 47,000 r.p.m. for 3 h at 18° C in an 'SW 50.1 rotor' in a gradient of 5 to 20% sucrose, NaCl 1 M, Tris 0.01 M, *p*H 8.0. ^{14}C-SV40 component I and II (21 and 16S) DNA were added as markers. The fractions were directly counted on glass filters. *a*, Native ^{3}H-DNA after the polymerase reaction; *b*, native ^{3}H-DNA before the polymerase reaction; *c*, heat-denatured (100° C, 10 min) ^{3}H-DNA after the polymerase reaction; *d*, heat-denatured (100° C, 10 min) ^{3}H-DNA before the polymerase reaction.

37° C overnight followed by two phenol extractions. The extracted DNA was precipitated with alcohol, dissolved in Tris buffer, 0.01 M, *p*H 7.2, and EDTA 0.01 M and treated with RNAase, 30 μg ml.$^{-1}$, free of DNAase for 1 h at 37° C, followed by pronase treatment (1 mg ml.$^{-1}$, 1 h at 37° C) and phenol extraction twice. The DNA was precipitated, washed with alcohol and dissolved in 0.0025 M EDTA, *p*H 7.2, and sonicated to reduce the size to $2–4\times10^5$ in its native form (determined as in Fig. 2). Cold EBV DNA was also sonicated to the same size. The heat-denatured Raji DNA (500 μg) was mixed with 1.4×10^5 c.p.m. (0.026 μg) of heat denatured ^{3}H-EBV DNA in 1 ml. of phosphate buffer, 0.09 M, *p*H 6.8 (0.135 M Na^+). This ratio of cellular DNA to EBV-DNA was equivalent to 2.6 genomes per cell[4] if cellular DNA does not contain any EBV genomes. We used HeLa DNA as a viscosity control; thus 500 μg of heat-denatured HeLa cell DNA was mixed with 1.4×10^5 c.p.m. of heat-denatured ^{3}H-EBV DNA (2.6 genomes/cell) or with 1.4×10^5 c.p.m. of heat-denatured ^{3}H-EBV DNA and 0.06 μg of cold EBV DNA (8.6 genomes/cell). Each glass tube containing 0.1 ml. of the mixture was sealed and incubated at 65° C for each period. The kinetics of reassociation follows the equation $C/C_0=1/1+K\,C_0t$[11] where C is the concentration of unreassociated DNA, C_0 is the initial concentration of DNA, t is time, and K is the reassociation constant. At each time indicated in Fig. 3, samples were taken and frozen at −20° C until all the reactions were finished. Single-stranded (unreassociated) and double-stranded (reassociated)

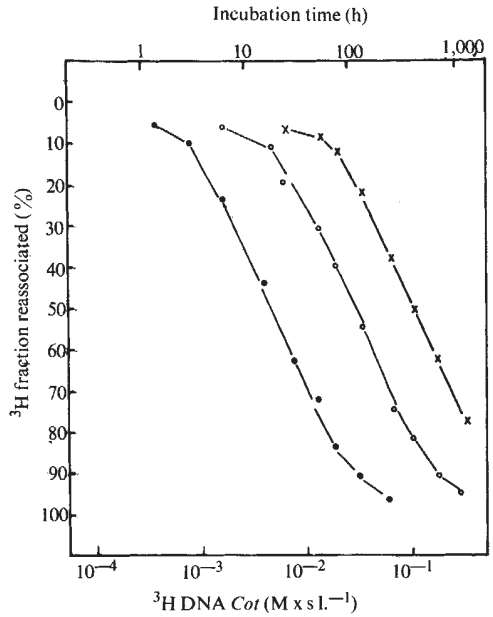

Fig. 3 DNA–DNA reassociation curves for the mixture of Raji DNA and ^{3}H-EBV DNA. 500 μg of sonicated and heat-denatured cellular DNA and heat-denatured EBV–DNA was mixed in 1 ml. of 0.09 M sodium phosphate buffer, *p*H 6.8, and incubated at 66° C for the indicated period. Single and double-stranded DNA were fractionated through a hydroxyapatite column at 60° C with 0.14 M phosphate buffer containing 0.4% SDS and 0.4 M phosphate buffer containing 0.4% SDS, respectively. ●, 500 μg of Raji DNA and 1.4×10^5 c.p.m. (0.026 μg, 2.6 genomes/cell) of ^{3}H-EBV DNA; ○, 500 μg of HeLa DNA and 1.4×10^5 c.p.m. (2.6 genomes/cell) of ^{3}H-EBV DNA and 0.06 μg (6 genomes/cell) of cold EBV DNA; ×, 500 μg of HeLa DNA and 1.4×10^5 c.p.m. (2.6 genomes/cell) of ^{3}H-EBV DNA.

DNAs were fractionated by hydroxyapatite chromatography (diameter, 1.2 cm ; height, 3 cm) at 60° C[5]. Single-stranded DNA was eluted by 0.14 M phosphate buffer with 0.4% SDS, and double-stranded DNA was eluted by 0.4 M phosphate buffer with 0.4% SDS. More than 95% of the DNA was eluted in each corresponding fraction when single and double-stranded DNA were chromatographed separately. The effluents were cooled to room temperature and precipitated with cold 5% TCA and counted in a liquid scintillation counter.

Results are shown in Fig. 3. Half C_0t values (50% reassociation) of ^{3}H-EBV DNA with 2.6 genomes/cell and 8.6 genomes/cell were 9.9×10^{-2} and 2.9×10^{-2} M × s l.$^{-1}$ respectively. Thus when the number of genomes was 3.3 times more, the reassociation went 3.4 times faster. The two values agreed well. The half C_0t value of ^{3}H-EBV DNA with Raji DNA was 4.7×10^{-3}. The reassociation occurred 6.2 times faster than with 8.6 genomes/cell and 21.0 times faster than with 2.6 genomes/cell. Thus the total number of EBV genomes present in the mixture of Raji DNA and ^{3}H-EBV DNA was 53.4, calculated from 8.6 genomes/cell and 54.6 genomes from 2.6 genomes/cell. After subtraction of 2.6 genomes of ^{3}H-EBV DNA, the number of EBV genomes present in Raji cells became 50.8 from 8.6 genomes/cell and 52.0 genomes/cell from 2.6 genomes/cell. We have repeated a cRNA hybridization determination for Raji DNA[4] and found 57 genomes/cell, compared with 50 genomes/cell reported by the cRNA method[3]. The number of EBV genomes obtained by association kinetics and by the cRNA method therefore did not show a significant discrepancy.

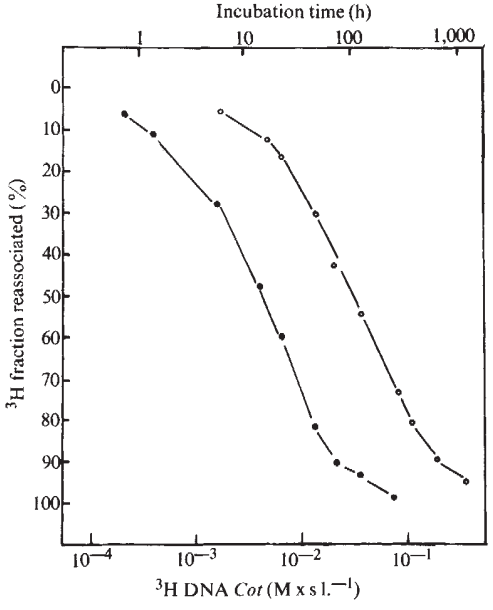

Fig. 4 DNA–DNA reassociation curves for a mixture of DNA from a biopsy of Burkitt's lymphoma and ^{3}H-EBV DNA. ●, 500 μg of biopsy DNA and 1.4×10^5 c.p.m. (0.026 μg, 2.6 genomes/cell) of ^{3}H-EBV DNA; ○, 500 μg of HeLa DNA and 1.4×10^5 c.p.m. of ^{3}H-EBV DNA (2.6 genomes/cell) and 0.06 μg of cold EBV DNA (6 genomes/cell).

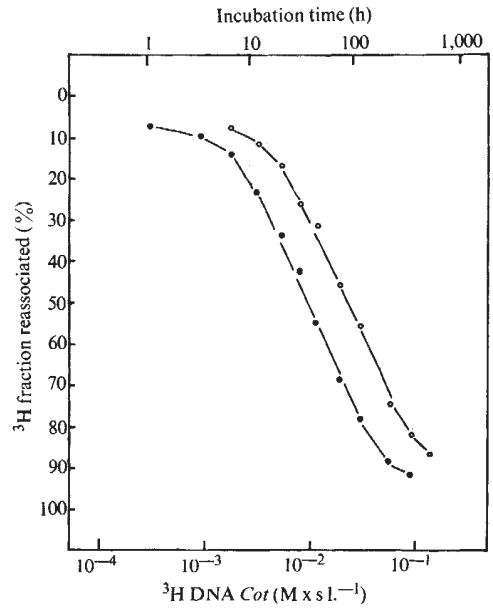

Fig. 5 DNA–DNA reassociation curves for a mixture of DNA from a biopsy of nasopharyngeal carcinoma and ^{3}H-EBV DNA. ●, 500 μg of biopsy DNA and 1.4×10^5 c.p.m. (0.026 μg, 2.6 genomes/cell) of ^{3}H-EBV DNA; ○, 500 μg of HeLa DNA and 1.4×10^5 c.p.m. of ^{3}H-EBV DNA (2.6 genomes/cell) and 0.06 μg of cold EBV DNA (6 genomes/cell).

We have obtained biopsies of Burkitt lymphoma (BL) and nasopharyngeal carcinoma tissue (NPC) (from Professor G. Klein, Stockholm), some of which yielded enough DNA to conduct reassociation kinetics experiments. Procedures were identical to the Raji experiment; the control experiment employing 8.6 genomes/cell was carried out simultaneously. Results are shown in Fig. 4 and Fig. 5. One of the BL biopsies had a half C_0t value of 4.3×10^{-3}; the 8.6 genomes/cell control had a value of 2.6×10^{-2} (Fig. 4). An NPC biopsy showed a half C_0t value of 1.1×10^{-2}; the 8.6 genomes/cell control had a value of 2.8×10^{-2} (Fig. 5). The BL biopsy from these figures contained 49.4 genomes/cell, and the NPC biopsy carried 19.2 genomes/cell; cRNA

hybridization resulted in 45 genomes/cell and 19 genomes/cell for the same biopsies. Reassociation curves for the DNA of Raji cells, BL biopsy and NPC biopsy showed a pattern identical to control EBV DNA and did not show a biphasic curve which is evidence for partial homology. Thus, Raji, BL biopsy and NPC biopsy contain complete or almost complete viral genomes. We believe this is the first evidence which indicates that the virus DNA in fresh NPC biopsies is not only immunologically related but identical to that in BL biopsies.

Here we have shown that the cRNA hybridization method gives essentially the same number of EBV genomes/cell as DNA–DNA reassociation kinetics. EBV DNA in Raji cells exists as free viral DNA under denaturing conditions[12] in contrast to SV40 DNA in transformed cells[13]. The reconstruction control experiment for cRNA hybridization in which HeLa DNA and EBV DNA are mixed would simulate the condition of EBV DNA in Raji cells[12]. Therefore any preferential overestimation or underestimation of viral DNA should not be expected.

cRNA hybridization is simple and fast for multiple sample analysis, but is not sensitive enough to detect less than two genomes/cell nor does it give the degree of homology. Reassociation kinetics is relatively slow, requiring larger amounts of cellular DNA, but is sensitive enough to detect as little as 0.2 genomes/cell. It is also of use to examine from the shape of the curve whether the viral DNA in biopsies from various sources is partially homologous or identical to EBV DNA, or whether complete or deleted genomes are present. A combination of these two methods and the *in situ* cytohybridization method[14] will be a great help for the study of biopsies related to latent viruses.

We thank Professor G. Klein for the gift of biopsies of Burkitt's lymphoma and nasopharyngeal carcinoma, and Mrs C. H. Huang for technical assistance. This study was conducted within the Special Virus-Cancer Program of the National Cancer Institute, NIH, PHS, and a grant from the John A. Hartford Foundation. J. S. P. holds a Research Cancer Development award.

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Received July 4; revised August 30, 1972.

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Cell Fusion induced by a Virus within the Zona Pellucida of Mouse Eggs

MAMMALIAN egg cells, like other cells, can be fused in culture in the presence of a virus[1,2]. But because many viruses do not infect eggs which possess a zona pellucida[3], fusion of egg cells has been performed after its removal. This manipulation reduces the rate of successful development in early cleavage eggs[4], which if transferred to recipient oviducts adhere to the epithelium and degenerate[5]. Now we report multinucleation and nuclear fusion within the zona pellucida of mouse eggs injected with a suspension of Sendai virus and somatic cells into the perivitelline cavity.

Sendai virus with a titre of $10^{7.3}$ 50% embryos lethal dose (ELD_{50}) ml.$^{-1}$ was inactivated by ultraviolet light[6] before diluting five-fold with sterile balanced salt solution[7]. Somatic cells from C57BL/6 mice were pipetted into the virus suspension and maintained at 5° C for 15–30 min. For micrurgical manipulation, two- or eight-celled eggs from superovulated BALB/c mice were deposited into a suspension of somatic cells and virus in an "egg-well"[8]. Two to six cervical lymph node[9] or femur bone marrow[1] cells with 5,000–10,000 μm^3 of the suspension were injected into the perivitelline cavity[8] by means of a bevelled micropipette. The micropipette was prepared by grinding at an angle of 35° under a dissecting microscope using a micromanipulator to set the tip on the edge of a water-sprinkled glass wheel, which was surfaced by a No. 600 carborundum and driven by a motor at 850 r.p.m. The pipette orifice, about 10 μm, is good for pipetting somatic cells for viable fusion[10]. It may, however, injure one-celled eggs. Late

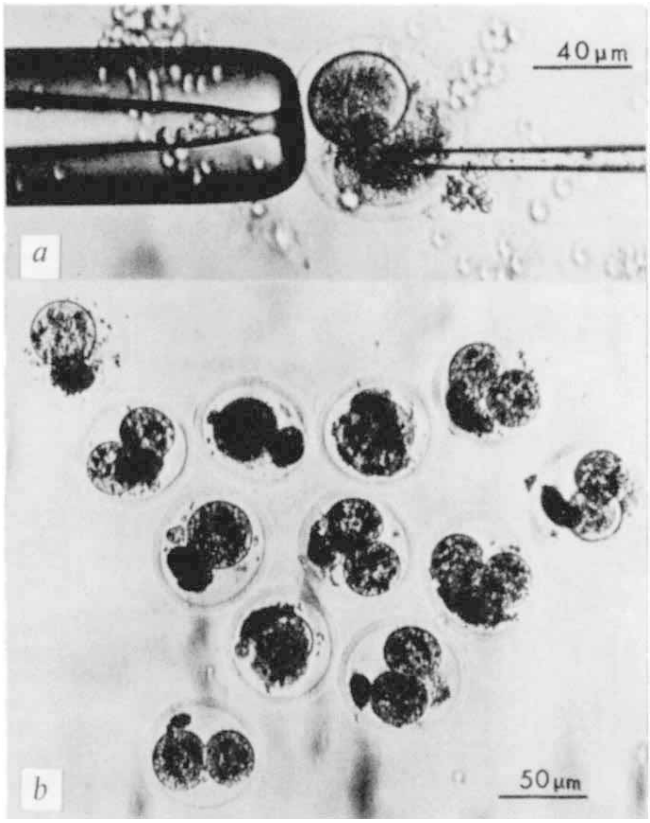

Fig. 1 Micrurgical manipulation of mouse eggs and their development in culture. *a*, A bevelled micropipette entered the two-celled egg and damaged one blastomere. *b*, A group of micrurgically manipulated two-celled eggs was injected with a suspension of Sendai virus and somatic cells and then cultured overnight. The manipulation destroyed one cell. Most of these eggs divided to a two-cell condition; some eggs did not divide (or fused). The unremoved remnant of the destroyed blastomeres in these eggs became a dense mass.

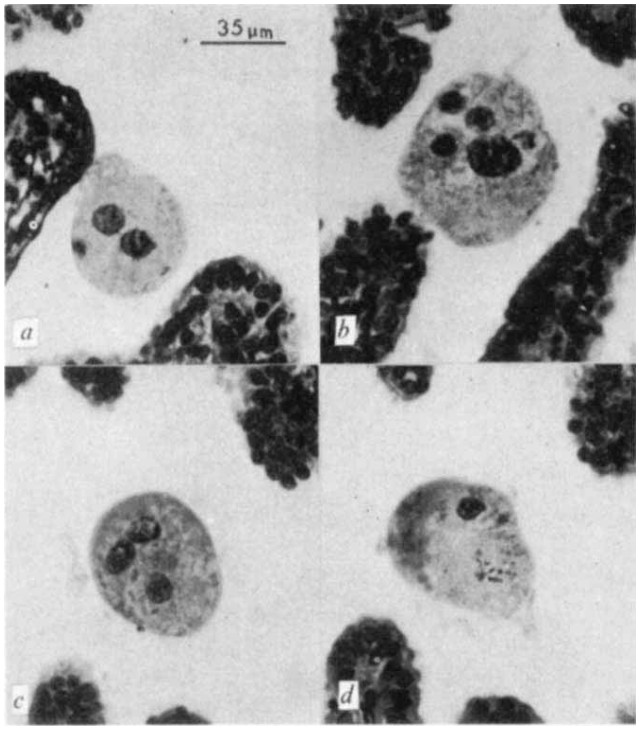

Fig. 2 Cell fusion in developing mouse eggs observed in sections of recipient oviducts. *a*, Originally a two-celled egg in which one blastomere was destroyed and partially removed before a suspension of Sendai virus and bone marrow cells was injected into the perivitelline cavity. The egg divided to restore the two-celled state and then fused in an overnight culture. The zona pellucida of the egg was dissolved in fixation. This section shows two nuclei derived from the fusion of two cells. A remnant of the destroyed blastomere can be seen on top of the fused, multinucleated cell. A somatic nucleus was located at the left edge of the cell. *b*, A section of a morula developed from a virus-injected eight-celled egg. Fusion of a few egg cells in this embryo is evident; the greater mass of nuclear material was apparently derived from the fusion of several nuclei. *c*, Another embryo with a large cell containing two nuclei. *d*, An alternate section of the same embryo showing one nucleus in mitotic metaphase.

two-celled eggs were therefore used after destruction or partial removal of one blastomere (Fig. 1*a*), leaving one viable egg cell[11]. In the eight-celled eggs one or two blastomeres were damaged during injection. Similarly treated eggs which received an injection of balanced salt solution served as controls. The eggs were then cultured at 37° C for two days and observed for division, degeneration, or fusion. Some eggs were incubated 3 h to study early cell fusion; other eggs were cultured 1 or 2 days and transferred to the oviducts[12] of virgin mice for fixation and serial microsectioning.

Table 1 Development of Mouse Eggs in Culture following Micrurgical Manipulation and Injection of Sendai Virus Cell Suspension

Egg stage at start	Egg changes	Eggs developing after an injection containing virus and cells (%) (experimental)* Day 1	Day 2	Eggs developing with injection of balanced salt solution (%) (control)† Day 1	Day 2
Two-celled (one cell destroyed)	Developing	56	39	57	59
	Degeneration	4	28	0	10
	No change	40	33	43	31
Eight-celled (one or two cells damaged)	Developing	74	52	92	72
	Degeneration	8	21	8	28
	No change	18	27	0	0

* Ninety two-celled eggs and sixty-six eight-celled eggs were injected with virus-cell suspension.

† Fifty-eight two-celled eggs and thirty-six eight-celled eggs were used for the controls.

Two- and eight-celled experimental and control eggs survived in culture (Table 1). After damaging one blastomere of the two-celled eggs, the remaining blastomere divided and restored a "two-celled" stage (Fig. 1*b*) which was equivalent to four-celled eggs if undamaged on the first day of culture. On the second day, many eggs developed to the "four-celled" stage, which normally would have been eight-celled. During culture, some divided two-celled eggs, injected with Sendai virus, fused into a single cell or degenerated more than the control eggs. Fusion of some virus-injected eggs could only be detected by microsectioning.

The eight-celled eggs, injected with the virus-cell suspension or with balanced salt solution (control), similarly developed to early morulae in overnight culture. Some virus-injected eggs developed to late morulae or blastocysts the next day. Blastomere fusion of the virus-injected eggs was also noted.

Serial microsections of nine oviducts were examined. Each received three to four eggs for a total of thirty-four two-celled eggs which had been injected with the virus and somatic cell suspension. Six of these eggs fused as evidenced by the presence of two nuclei (Fig. 2*a*). The presence of a somatic nucleus in an egg was rarely observed. Many somatic cells adhered to the vitelline surface of the eggs after 3 h in culture.

Eight out of eighteen virus-injected eight-celled eggs showed fusion as located in the microsections of five recipient oviducts. Fusion between introduced somatic cells and egg cells inside the zona pellucida was less frequent than fusion between egg cells themselves.

When fusion occurred in "two-celled" eggs within the zona pellucida, it produced a single cell (Fig. 2*a*), whereas in morulae developed from eight celled eggs, only a few cells of the embryos fused (Fig. 2*b* and *c*). During multinucleation, two or more nuclei of egg cells often fused to create a nucleus of greater mass within an enlarged egg cell (Fig. 2*b*). The partial fusion and multinucleation in morulae occurred frequently, producing cytologically unbalanced mosaic preimplantation embryos. Some of the embryonic cells were actively dividing (Fig. 2*d*).

In several preliminary experiments, we have micropipetted a virus-treated lymphocyte or an erythroblast into the perivitelline space to attempt fusion with the vitellus of unfertilized mouse eggs. Sometimes the two cells fused but the somatic cell nucleus did not always reach the middle of the ooplasm. In amphibians, small blastomeres[13] have been used more successfully than embryonic intestinal cells[14] in the nuclear transfer to an unfertilized ovum for development. Our results would indicate that, in the mouse, nuclear cloning of unfertilized eggs with somatic cells by means of virus fusion may not be as easy as with egg cells or small blastomeres.

We thank Drs J. J. Elias and R. H. Glass for reviewing the manuscript, and Miss Michiko Kasahara, Mrs Nancy Schlenke and Mr D. R. Akers for technical assistance. This work was supported by grants from the US National Institutes of Health.

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Antibody Induced Variation in Malaria Parasites

SUCCESSFUL cellular differentiation and function are dependent upon responsiveness to external stimuli, both useful and harmful, and this responsiveness is particularly evident among some parasitic protozoa. Their environment changes abruptly at transmission from invertebrate vector to mammalian host and becomes potentially harmful when the host mounts an immune response. Several, and possibly most, protozoan parasites avoid total destruction by the immune response they evoke by repeated changes of antigenicity[1]. Replacement of one population by another is detectable in tests carried out at weekly intervals[2,3], and variation at this rate apparently continues for months and perhaps even years. In the absence of suitable techniques for *in vitro* cultivation, the question remained whether this variation resulted from immunoselection or from a form of antigenic modulation. Here I have attempted to clarify this point with one species of malaria parasite, *Plasmodium knowlesi*, using an *in vivo* technique based upon earlier observations[4,5] that *Macaca mulatta*, sensitized with *P. knowlesi* antigen in incomplete Freund's adjuvant, produces high titres of variant-specific schizont-infected cell agglutinating antibodies which were not protective. Animals sensitized in this way were challenged with homologous parasites in numbers small enough to allow detection of possible immunoselection by delay in parasitaemia or failure in the appearance of a new antigenic variant. Results indicated that antigenic variation in *P. knowlesi* is non-selective and that potential for variation on this scale is an integral part of the parasite genome. Three experiments were carried out with similar results, and one is described here.

Two *Macaca mulatta*, approximately 4 kg, were infected with *P. knowlesi* obtained from a frozen stabilate. When the parasitaemias exceeded 30%, schizont-infected cells were harvested for preparation of freeze-thawed *P. knowlesi* antigen, and aliquots of the infected cells were also frozen as a source of stabilate material for challenging sensitized monkeys. Monkeys weighing 2.5 to 4.0 kg were then sensitized with the *P. knowlesi* antigen in incomplete Freund's adjuvant as described previously[4]. After sensitization, they were divided randomly into three groups each of three animals; serum collected at this time from all animals gave a reciprocal schizont-infected cell agglutinin (SICA) titre greater than 10^5. Three groups of three unsensitized animals served as controls; their sera gave reciprocal SICA titres of less than ten.

An additional monkey was infected with stabilate material isolated at the time of antigen collection, to provide homologous parasites for challenge. At a time when "ring stage" parasites predominated, blood was collected into cold heparinized TC 199 medium containing 5% normal monkey serum, parasitized and total red cells counted, and the blood diluted to the required concentration in the same medium. Groups of sensitized and control monkeys were inoculated intravenously with dilutions equivalent to 10^3, 10^2 or 10 parasitized cells, and their infections monitored by daily blood films. The results are given in Fig. 1.

Schizont-infected cells were isolated for SICA tests, either directly from the challenged monkeys or from normal monkeys

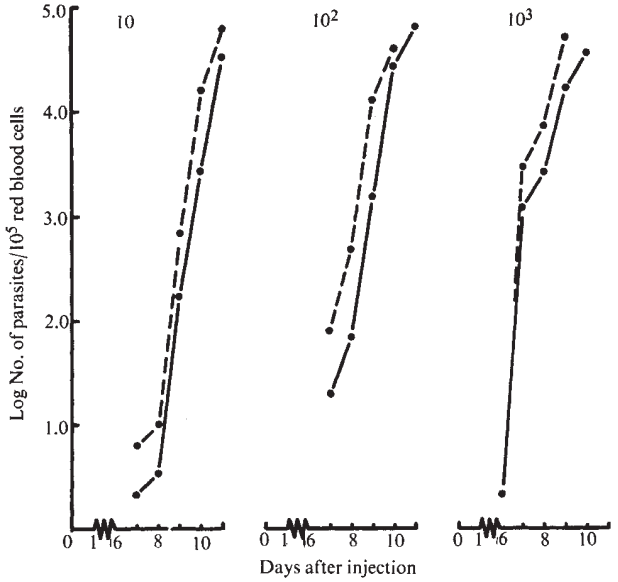

Fig. 1 Geometric mean parasitaemias in groups of *M. mulatta* inoculated with about 10, 10^2 or 10^3 erythrocytes infected with "ring stage" *P. knowlesi*. Three monkeys in each group. ●——●, Sensitized monkeys; ●------●, controls.

receiving an infective inoculum from a challenged animal. The serotypes as determined by SICA tests, using parasites from one monkey in each of the groups receiving 10 or 100 parasites, appear in Table 1; schizont-infected cells were tested against prechallenge sera from both the pre-sensitized or control parasite donors in each group.

Table 1 Schizont-infected Cell Agglutination Tests

Challenge inoculum	Schizont-infected cell donor	Serum donor	Reciprocal log titre
circ. 10	Sensitized	Sensitized	<1.0
		Control	<1.0
		Immune*	5.2
	Control	Sensitized	5.8
		Control	<1.0
		Immune*	<5.8
circ. 100	Sensitized	Sensitized	<1.0
		Control	<1.0
		Immune*	<5.8
	Control	Sensitized	<5.8
		Control	<1.0
		Immune*	<5.8

* Monkey immune after prolonged chronic infection—positive control.

A slight delay of less than 0.5 day in the development of the parasitaemia was detectable in the 3 groups of sensitized animals. This small difference occurred consistently in the three experiments; it was never greater than 0.5 day and was not related to the order in which the monkeys were inoculated. If this delay was due to destruction of parasites in sensitized animals, then comparison of the parasitaemia in sensitized animals receiving 10^2 with controls receiving 10 parasitized cells, and sensitized animals receiving 10^3 parasitized cells with controls receiving 10^2 cells, showed that over half the parasites inoculated into sensitized animals must have survived. Yet this high survival rate was consistently associated in all groups with a complete change of serotype in the sensitized animals and absence of change in the controls (Table 1). Thus the serotype change was apparently not an immunoselective process, but an antibody-induced change in antigen synthesis. The very slight delay in the parasitaemia apparent in the sensitized animals may in fact indicate not destruction of parasites but a

short check in maturation associated with the antigenic change. A more extended delay has been reported in monkeys sensitized with a succession of variants[6].

Variation of the type induced by this *in vivo* technique occurs repeatedly in chronically infected animals and appears analogous to that occurring in free-living ciliates[7]. The stage in the parasite cell cycle at which suitable antibodies can trigger antigenic modification is unknown, as are the molecular mechanisms involved, but analogy with free-living Paramecium suggests that the genome of the malaria parasite carries information for the synthesis of many alternative antigens, presumably with similar function.

Current experiments indicate that the degree of protective immunity shown by a host to malaria infection depends on the relative rate of synthesis of antigen modifying antibodies of the type demonstrated here, and parasiticidal antibodies of equal specificity capable of destroying parasites before they change the appropriate antigens. Increases in the rate of synthesis of parasiticidal antibodies compared with variation-inducing antibodies apparently correlate with greater parasite destruction and thus more protective immunity.

The possibility has been discussed elsewhere[1] that antigenic variation in protozoa might provide a valuable model for other situations where pathogenic cells persist in an apparently immunologically adverse environment. Phenotypic regulation by specific immunoglobulins may also prove to be of importance in non-pathogenic phenomena, as has been suggested in lymphoid cell maturation[8] and avoidance of foetal rejection[9], and possibly represents one way in which phenoclones[10] of cells may arise in development; their action may sometimes involve a degree of sublethal damage[7,11]. Protozoa are useful models for the interaction of environment and genome, both at the molecular and the organelle level[12]. They display in a readily detectable form responsiveness to environmental effects, including specific immunoglobulin binding, less easily observed but none the less important, in other cells at certain crucial stages in their differentiation.

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Received September 27, 1972.

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Transmission of the Chelonian Haemoproteid *Haemoproteus metchnikovi* by a Tabanid Fly *Chrysops callidus*

Haemoproteus metchnikovi (Simond, 1901), also referred to by Garnham[1] as *Simondia metchnikovi*, is a parasite of chelonians. For a number of years we have studied haematophagous insects that feed on turtles in an attempt to discover the mode of transmission of *H. metchnikovi*. Insects were collected, dissected and examined for the presence of intermediate developmental stages and sporozoites. During these studies a sporozoite was found in the salivary glands of the fly *Chrysops callidus*[2] (Diptera: Tabanidae), which gave rise to typical gametocytes of *H. metchnikovi* in erythrocytes when inoculated into laboratory raised turtles, *Chrysemys picta*. We believe this is the first report of a dipteran of the family Tabanidae serving as the intermediate host of a haemosporidian.

Our experimental field site was a pond, located in Oakland County, Michigan, with a natural population of the infected turtle host (*C. picta*) and fly intermediate host (*C. Callidus*). Adult *C. callidus* females were collected as they fed on bait turtles. These flies were dissected, and salivary glands removed and examined microscopically for the presence of sporozoites. Infected glands were placed in a small quantity of 0.75 M saline, broken to release the sporozoites and injected into the peritoneal cavity of laboratory raised *C. picta*. These had been obtained newly hatched and were established and maintained in the laboratory for three and a half to four and a half years, during which time periodic examinations of blood smears were made to ensure they were blood negative for haematozoa. All turtles inoculated with sporozoites became blood positive with gametocytes of *H. metchnikovi*. Our data indicated gametocyte trophozoites were first detectable in erythrocytes approximately 30 to 32 days after inoculation. They grew slowly and mature gametocytes were present in small numbers after approximately three months. We are now studying gametocyte development to maturity within the erythrocyte under laboratory conditions. Because experimental infection of turtles was only recently possible, the sequence of development of the parasite from sporozoite to mature gametocyte in the turtle has not been studied. DeGiusti[3] reported the presence of the megaloschizont in the turtle spleen, but with frozen sporozoite material now available, we are conducting experimental studies which should elucidate the development of the tissue phases within the turtle host.

Table 1 Natural Infection Rate of *C. callidus* with *H. metchnikovi* Sporozoites

	No. flies examined	No. flies infected	% infected
1971:			
June	166	18	11
July	81	23	28
1972:			
June	12	1	8
July	84	32	38
August	44	20	45

The sporozoites of *H. metchnikovi* were present in great numbers (> 1,000) in the salivary glands of *C. callidus*; one or both glands might be infected, and in some heavy infections the sporozoites completely filled the gland lumen. The sporozoites were crescent-like in shape and sometimes recurved on themselves forming short spirals. Motion was limited to sluggish flexing of the body without progressive activity. In smears prepared by drying and methyl alcohol fixation or combined osmic acid-methyl alcohol fixation, the sporozoites appeared as crescent-shaped bodies with one blunt and one tapered end (Fig. 1). In the majority of specimens studied the nucleus is subcentrally located as a single compact mass or split into two or more portions (Fig. 1). Fixed, stained sporozoites measured 9–12 μm in length and approximately 1–1.4 μm in width at their widest dimension.

Oocysts observed in tissue sections of the midgut wall of *C. callidus* were 10–18 μm in diameter. As we do not have experimentally infected flies at present, it is difficult to assess

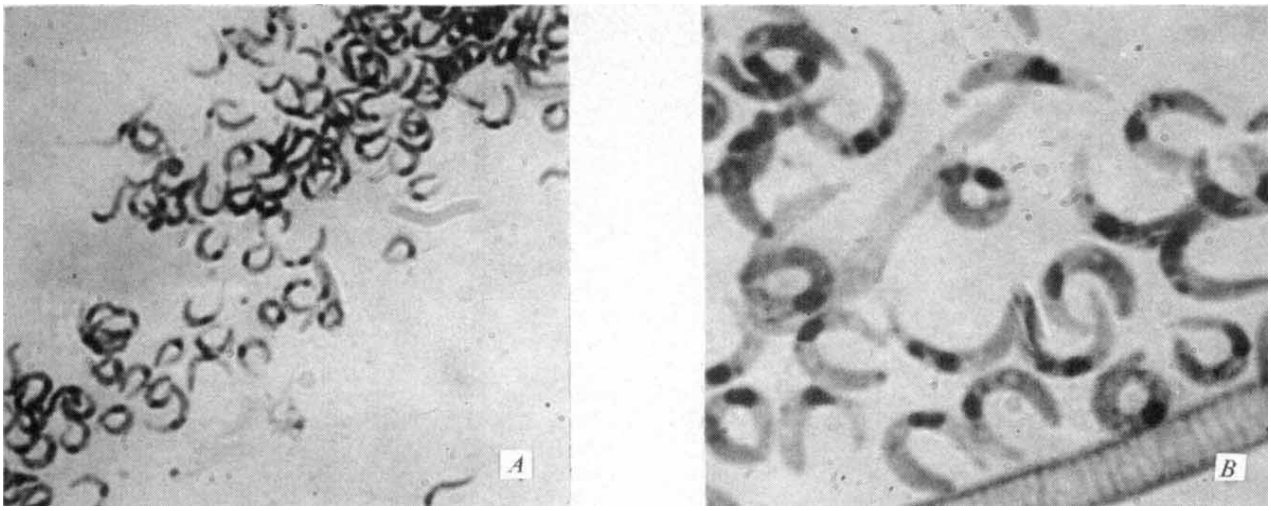

Fig. 1 Crush preparation of *C. callidus* salivary gland showing *H. metchnikovi* sporozoites (*A*, ×1,000; *B*, ×2,560).

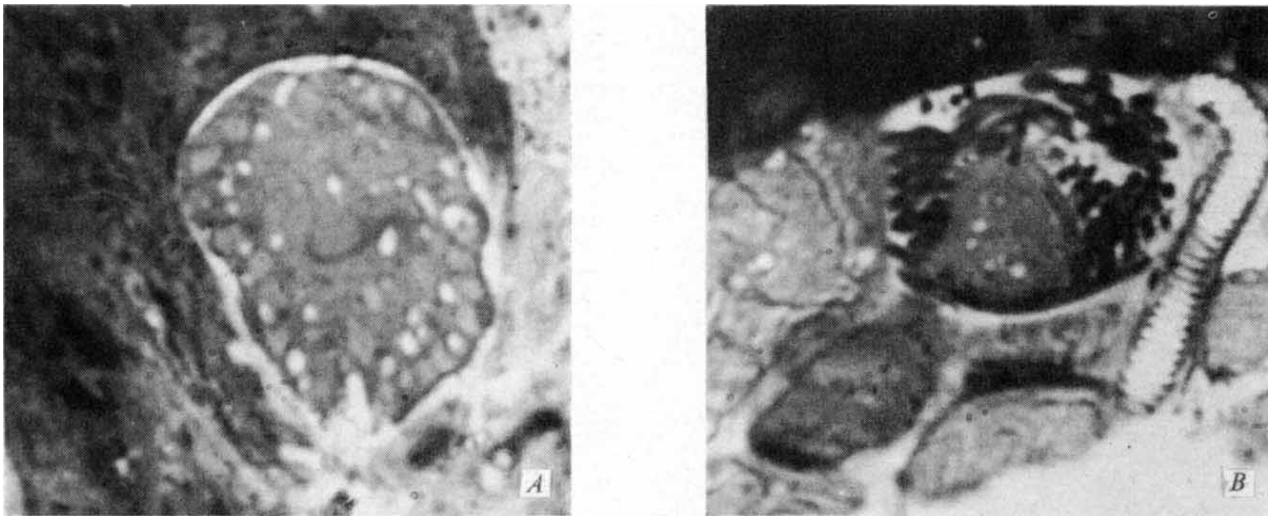

Fig. 2 Oocysts in midgut wall of *C. callidus*. *A*, Young oocyst; *B*, older oocyst with developing sporozoites. ×2,560.

the developmental stages of the oocyst. Those seen range from young stages (Fig. 2*A*) to stages with formed sporozoites (Fig. 2*B*).

Our field studies indicated the season of transmission may be relatively short, as can be seen from the data in Table 1. The earliest fly infections were encountered on June 22, and increased during July and early August, but by mid-August it became difficult to capture or sight flies on turtles, leading us tentatively to conclude that at this time the height of transmission had been reached. Salivary glands examined during this period showed evidence of sporozoite exhaustion; some contained as few as one to five sporozoites, supporting this conclusion. Little is known of the bionomics of *C. callidus*, but the information available indicates that in more southern areas of the US, such as Florida, the flies are active as adults in late April through June[4]. It is quite likely that in a northern area such as Michigan, the adult flies may be active from late May through early September.

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Received September 21, 1972.

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Tracing of RNA from a Puff in the Polytene Chromosomes to the Cytoplasm in *Chironomus tentans* Salivary Gland Cells

The transport of RNA from the cell nucleus to the cytoplasm is likely to be a composite process, the details of which are far from clear. There is a large discrepancy in molecular size between polysomal mRNA and its tentative precursor, the high molecular-weight, non-ribosomal, nuclear RNA (H RNA)[1]. Also, a considerable part of H RNA does not enter the cytoplasm, but is degraded to acid-soluble products within the nucleus[1]. To explain these findings it has been suggested that H RNA is cleaved to smaller molecules, among which mRNA sequences are selected for transfer to the cytoplasm[2]. In the

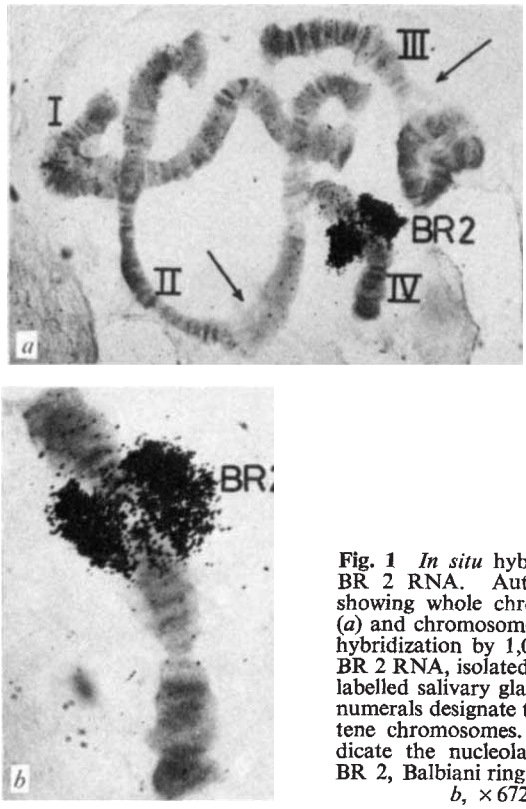

Fig. 1 *In situ* hybridization by BR 2 RNA. Autoradiographs showing whole chromosome set (*a*) and chromosome IV (*b*) after hybridization by 1,000 c.p.m. of BR 2 RNA, isolated from *in vitro* labelled salivary glands. Roman numerals designate the four polytene chromosomes. Arrows indicate the nucleolar organizers. BR 2, Balbiani ring 2. *a*, ×252; *b*, ×672.

study of such transport phenomena it would be advantageous to follow a specific RNA from its site of synthesis in the chromosomes to its functional locality in the cytoplasm. This possibility may be offered in the study of RNA from the giant puff Balbiani ring 2 (BR 2) in the polytene chromosomes of *Chironomus tentans* salivary glands.

Cytogenetic and biochemical studies[3–5] of salivary glands in larvae of Chironomus species have provided a correlation between synthetic activity in the Balbiani rings and the appearance of specific protein fractions in the salivary secretion. It has been suggested that Balbiani rings may produce mRNA for the secretory proteins[4], but this has not been directly demonstrated. BR 2 RNA is present in its corresponding puff as a large molecule with a sedimentation value of approximately 75 S, and appears undegraded in the nuclear sap[6]. Electron micrographs of Balbiani rings reveal characteristic granules of about 400–500 Å in size, which can be traced through the nuclear sap into the pores of the nuclear envelope[7,8]. Although it seems likely, on the basis of these results, that BR RNA does enter the cytoplasm, it has not been possible to show yet. Cytological hybridization may prove to be valuable in this respect, because BR 2 RNA hybridizes specifically *in situ* with its corresponding chromosomal puff[9,10]. Here I have taken advantage of this specificity of BR 2 RNA/DNA hybridization *in situ* to investigate the transfer of BR 2 RNA into the cytoplasm. Labelled RNA was extracted from Balbiani rings, from the nuclear sap surrounding the polytene chromosomes and from the peripheral cytoplasm, and hybridized to denatured squash preparations of salivary gland cells. The location of the ribonuclease-resistant RNA/DNA hybrids was studied by autoradiography.

Balbiani rings and nuclear sap were isolated by microdissection of salivary glands which had been subjected to *in vitro* labelling for 90 min with 100+100 μCi of tritiated uridine plus cytidine in 50 μl. of modified 'Cannons insect medium'[9]. Cytoplasm was micro-dissected from salivary glands of larvae which had been living for 7 days in ordinary rearing medium supplemented with 1 mCi ml.$^{-1}$ of tritiated uridine and cytidine. Only the peripheral cytoplasm was used, so that in the micro-manipulatory step the nuclei were left intact with a distinct zone of surrounding cytoplasm in order to avoid nuclear contamination, and only the peripheral zone of cytoplasm was collected.

The labelled RNA was liberated by dissolution of the microdissected samples in droplets of Tris buffer (*p*H 7.4) containing pronase (1 mg ml.$^{-1}$) and sodium dodecyl sulphate (5 mg ml.$^{-1}$). The droplets were incubated for 3 h in 37° C, and then diluted with 75 μl. of 2×SSC. This solution was heated for 5 min at 100° C to reduce the molecular size of the RNA[9]. Squashes of salivary gland cells were made and treated with ribonuclease. Denaturation of DNA *in situ* was accomplished by treatment of the slides in 90% formamide in 0.1×SSC at 63° C for 2.5 h. Ten μl. of the labelled RNA in 2×SSC was added to each squash preparation, which was then covered by a cover-slip and incubated for 4 h at 63° C in sealed Petri dishes. The hybridization was interrupted by immersing the slides in ice-cold 2×SSC. The slides were subsequently treated with ribonuclease (100 μg ml.$^{-1}$ of 2×SSC, 2 h at 37° C), and extensively washed in 2×SSC. Autoradiographs were prepared with 'Kodak AR 10' stripping film, and the slides were exposed for 2 months.

Preparations challenged with BR 2 RNA showed distinct labelling in the BR 2 region of chromosome IV, and comparatively few grains were seen outside this region (Fig. 1*a* and *b*). The nucleolar organizers were not labelled (Fig. 1*a*). Neither RNA from chromosomes I, II or III, nor nucleolar RNA, gave rise to a significant number of grains in the BR 2 region[9]. Consequently, *in situ* hybridization with BR 2 RNA is specific in labelling only its corresponding puff region. The heavy labelling over BR 2 in these preparations indicates that BR 2 contains repeated DNA sequences. A similar conclusion was drawn from analyses of quantitative and kinetic hybridizations between BR 2 RNA and filterbound *Chironomus tentans* DNA[10].

Nuclear sap RNA hybridized in a fashion similar to that of BR 2 RNA, the difference being mainly a slightly higher number of grains over the chromosomes (Fig. 2*a*). The BR 2 region of chromosome IV was heavily labelled (Fig. 2*b*) which indicates the presence of BR 2 RNA in the nuclear sap. This result agrees with previous experiments[11,12] in which the proportion of BR 2 RNA out of total, newly synthesized, nuclear sap H RNA was estimated to be at least 50%.

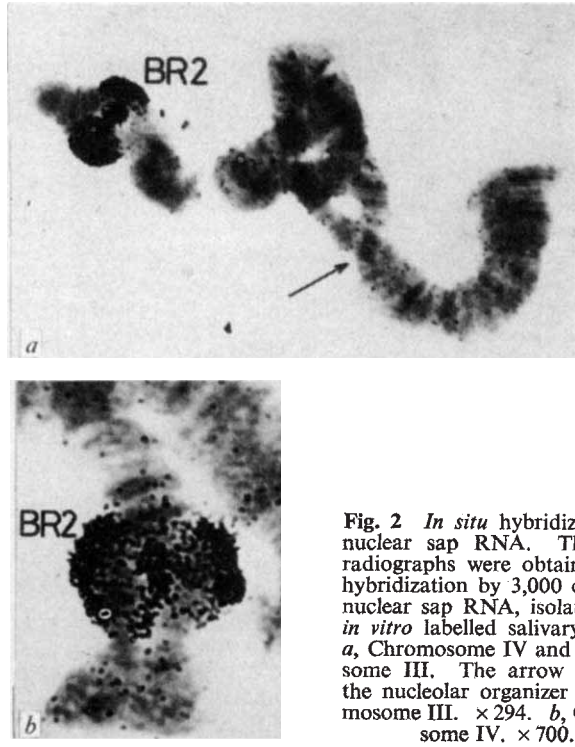

Fig. 2 *In situ* hybridization by nuclear sap RNA. The autoradiographs were obtained after hybridization by 3,000 c.p.m. of nuclear sap RNA, isolated from *in vitro* labelled salivary glands. *a*, Chromosome IV and chromosome III. The arrow indicates the nucleolar organizer of chromosome III. ×294. *b*, Chromosome IV. ×700.

In the preparations hybridized with cytoplasmic RNA, most of the grains were again found in the BR 2 region of chromosome IV (Fig. 3), but a weak labelling was frequently observed also in the nucleolar organizers (Fig. 3*a*). An additional large puff on chromosome IV, Balbiani ring 1, was labelled, although with fewer grains than BR 2 (Fig. 3*b*). The finding of grains over the nucleolar organizers after hybridization with cytoplasmic RNA (Fig. 3*a*) but not after hybridization with nuclear sap RNA (Fig. 2*a*) was expected. Labelled ribosomal RNA accumulates in the cytoplasm during a long-time *in vivo* labelling[13], but enters the nuclear sap only to a very small extent during the 90 min *in vitro* labelling[13].

The number of grains in the BR 2 region after hybridization with cytoplasmic RNA (Fig. 3*b*) is significant, but smaller than after hybridization with *in vitro* labelled BR 2 RNA or nuclear sap RNA. This may be explained by the smaller isotope concentration used in the *in vivo* labelling of larvae, which will result in RNA with a lower specific activity. In addition to this, cytoplasmic dilution by pre-existing unlabelled BR 2 RNA might have taken place. Nevertheless the significant labelling in the BR 2 region obtained after hybridization with cytoplasmic RNA clearly shows that BR 2 RNA is present in the cytoplasm. In control experiments run in parallel to those shown in Fig. 3, it was found that RNA, extracted from the nuclei of those *in vivo* labelled glands which had been used for cytoplasmic extraction, failed to give a significant number of grains over the chromosomes and Balbiani rings. This probably means that the amount of intranuclear, *in vivo* labelled BR 2 RNA is too small to be detected by the technique. Consequently, the positive results by using the cytoplasmic RNA suggest that an accumulation of BR 2 RNA takes place in the cytoplasm during the 7 days *in vivo* labelling.

The significant labelling in the BR 1 region (Fig. 3*b*) after hybridization with cytoplasmic RNA indicates that RNA from this puff is also present in the cytoplasm. In a previous study[10] it was shown that BR 1 RNA is able to hybridize with its corresponding puff in a way very reminiscent of BR 2 RNA hybridization, although the number of grains over BR 1 was always smaller. On the basis of those results it was suggested that BR 1 also contains repetitive DNA[10]. Label in the BR 1 region has also been observed after hybridization with nuclear sap RNA, although this is an infrequent finding. This variability could be related to biological differences between animals, because BR 1 is not always apparent, and it seldom reaches the size of BR 2. In the present experiments nuclear sap and cytoplasmic RNA came from different animals.

My demonstration of Balbiani ring RNA in the cytoplasm extends the previous line of ultrastructural[7,8] and biochemical[6,9,11] evidence, suggesting a peripheral transport and possible involvement of BR RNA in translational processes. The presence of BR RNA in the polysomes, however, remains to be shown, as well as its further processing in the cytoplasm.

This report was supported by grants from the Swedish Cancer Society and from Karolinska Institutet. I thank B. Sperens for technical assistance.

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Received September 22, 1972.

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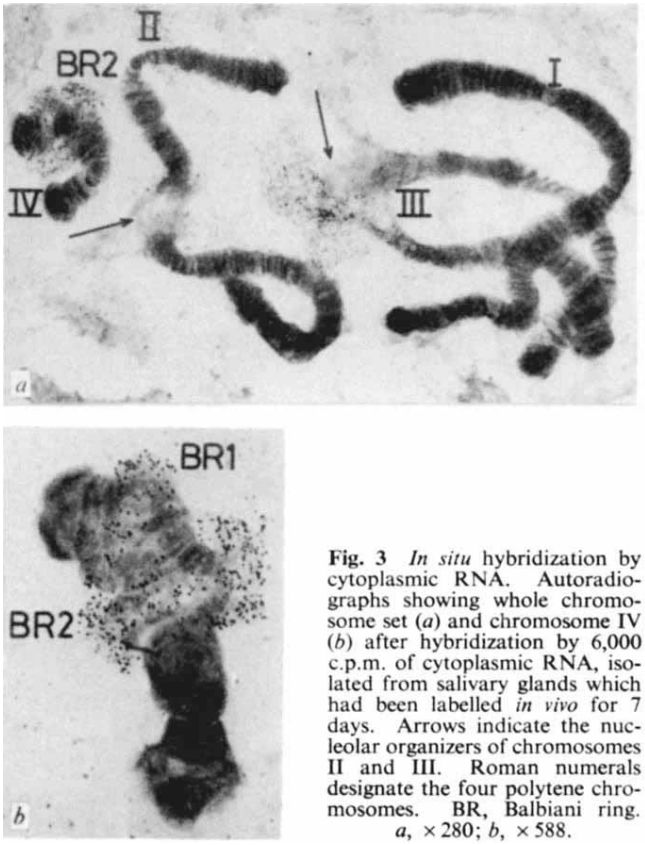

Fig. 3 *In situ* hybridization by cytoplasmic RNA. Autoradiographs showing whole chromosome set (*a*) and chromosome IV (*b*) after hybridization by 6,000 c.p.m. of cytoplasmic RNA, isolated from salivary glands which had been labelled *in vivo* for 7 days. Arrows indicate the nucleolar organizers of chromosomes II and III. Roman numerals designate the four polytene chromosomes. BR, Balbiani ring. *a*, ×280; *b*, ×588.

Conversion of the Sex Pheromone of the Cabbage Looper

THE mechanisms by which airborne molecules stimulate the olfactory receptors of insects remain largely undefined. In the Insecta, antennal sensilla contain pores which connect the external environment with the receptor membrane of the dendritic nerve endings[1–3]. The ultimate fate of the stimulant molecule that enters the pore is not yet known. Unpublished data cited by Schneider[1] and Kaissling[4] indicated that bombykol (E)-10, (Z)-12-hexadecadien-1-ol, was progressively metabolized into acid and ester after absorption on the antennae, or other body parts of males and females of *Bombyx mori* (L.). This finding cannot be directly correlated with any specific step in the olfactory mechanism, but it suggested that an enzymatic process might be involved at some point. Other indications of chemical stimulants interacting with protein substances in the antennae of insects have been reported[5,6].

We report a protein binding process and an enzymatic process in antennal homogenates of the cabbage looper, *Trichoplusia ni* (Hübner), which may be involved in the perception of pheromone. The cabbage looper was chosen for several cogent reasons: (*a*) the behavioural responses to the sex attractant ((Z)-7-dodecen-1-ol acetate) and various related chemicals have been well studied[7,8]; (*b*) electrophysiological studies have been completed with all the chemicals used here (to be reported); and (*c*) all chemicals and pheromones were available in high purity (95% + purity; the synthetic pheromone sample contained no measurable (Z)-7-dodecen-1-ol by GLC analysis).

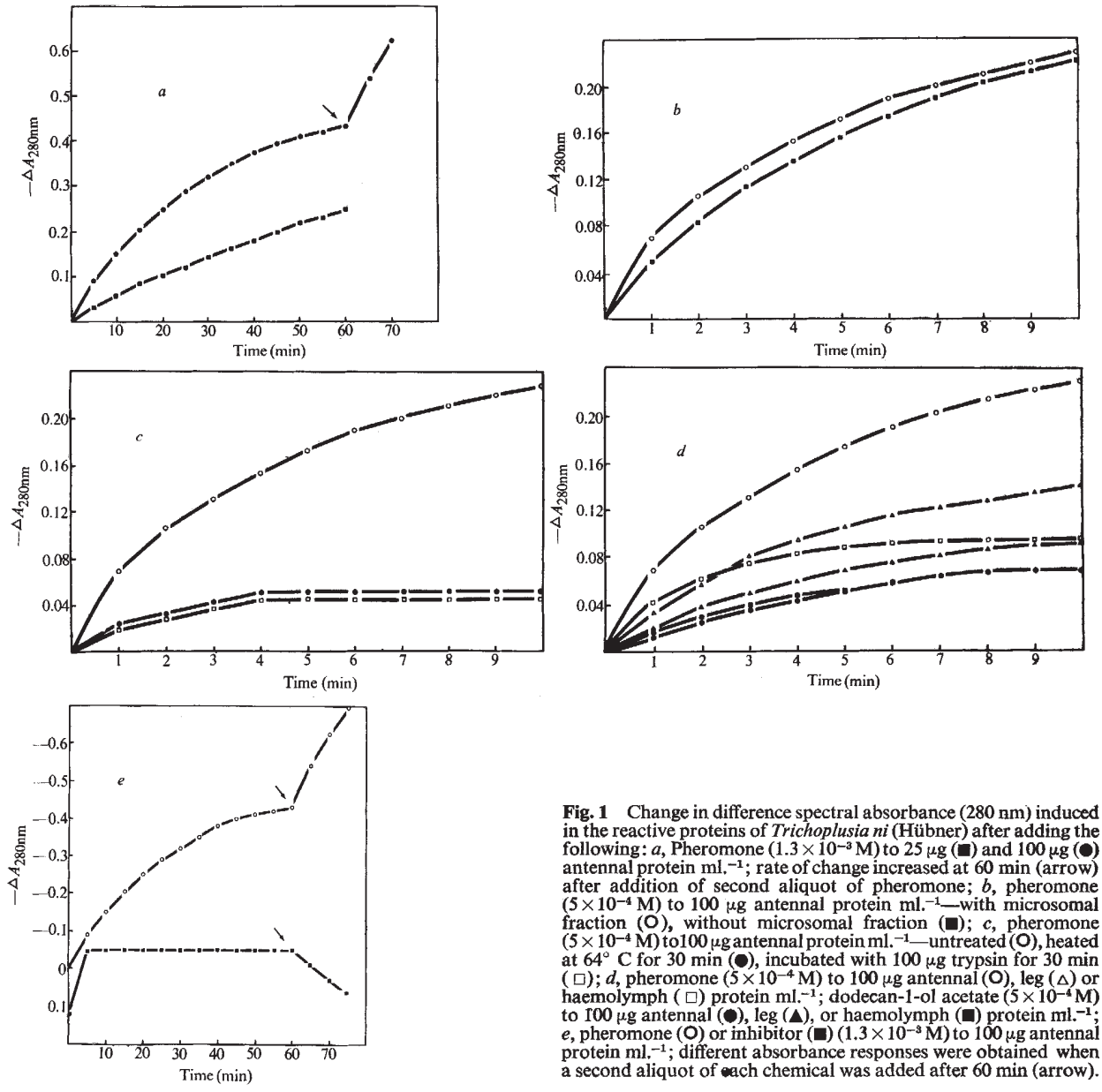

Fig. 1 Change in difference spectral absorbance (280 nm) induced in the reactive proteins of *Trichoplusia ni* (Hübner) after adding the following: *a*, Pheromone (1.3×10^{-3} M) to 25 μg (■) and 100 μg (●) antennal protein ml.$^{-1}$; rate of change increased at 60 min (arrow) after addition of second aliquot of pheromone; *b*, pheromone (5×10^{-4} M) to 100 μg antennal protein ml.$^{-1}$—with microsomal fraction (○), without microsomal fraction (■); *c*, pheromone (5×10^{-4} M) to100 μg antennal protein ml.$^{-1}$—untreated (○), heated at 64° C for 30 min (●), incubated with 100 μg trypsin for 30 min (□); *d*, pheromone (5×10^{-4} M) to 100 μg antennal (○), leg (△) or haemolymph (□) protein ml.$^{-1}$; dodecan-1-ol acetate (5×10^{-4} M) to 100 μg antennal (●), leg (▲), or haemolymph (■) protein ml.$^{-1}$; *e*, pheromone (○) or inhibitor (■) (1.3×10^{-3} M) to 100 μg antennal protein ml.$^{-1}$; different absorbance responses were obtained when a second aliquot of each chemical was added after 60 min (arrow).

Antennae from male moths (400 pairs, approximately 25 mg wet weight) were homogenized in 4.0 ml. of 0.5 M sucrose buffered with 0.05 M Tris-HCl, *p*H 7.5 at 4° C. The homogenate was centrifuged at 20,000*g* for 45 min and the supernatant was decanted. The protein content was determined by the method of Lowry *et al.*[9].

Binding of the sex attractant and its analogues to soluble antennal protein(s) was measured by ultraviolet difference spectroscopy[6,10,11]. Difference spectra recorded from 220 to 350 nm on a dual-beam recording ultraviolet-visible spectrophotometer, obtained with the antennal supernatant, the pheromone, and the pheromone analogues, indicated a time-dependent maximal negative peak at 280 nm. Subsequent measurements of the negative absorbance difference ($-\Delta A$ or negative change in optical density units) relative to the baseline at 280 nm were recorded with a single-beam ultraviolet-visible spectrophotometer. Baselines (zero absorbance (optical density) as a function of 280 nm) were attained by electronically neutralizing the original absorbance of the antennal preparation and the chemical stimulants with the absorbance control system (total absorbance negated was less than 1.0 OD unit). Full scale sensitivity of the recording system was set at 0–0.5 A, and the temperature of the cell compartment was thermostated at 20° C.

Twenty-five μl. of a sonicated solution of the pheromone in distilled water (final concentration 1.3×10^{-3} M) was added to 25 μg of antennal protein in 1 ml. of 0.05 M Tris-HCl, *p*H 7.5. The rate of absorbance change ($-\Delta A$) at 280 nm was dependent on the concentration of the antennal protein (Fig. 1*a*). As the concentration of protein was increased, the rate of change in absorbance also increased. After 1 h, the rate of absorbance change had diminished, but was reactivated by addition of another 25 μl. of pheromone.

A 2 ml. aliquot of the antennal supernatant was centrifuged at 105,000*g* for 2 h to remove the microsomes. The resulting particle-free supernatant reacted with the pheromone (5×10^{-4} M) with only a slight loss in activity (Fig. 1*b*). The activity of the antennal supernatant, however, was destroyed by heat at 64° C or incubation with 100 μg of trypsin (*p*H 7.5) at 20° C for 20 min (Fig. 1*c*). These results demonstrated that the monitored absorbance change involved interaction of the pheromone with soluble protein in the antennae. This suggests the interaction was enzymatic because the gradual change in absorbance was more characteristic of an enzymatic reaction than of nonenzymatic binding[10]. If the absorbance change had been due only to pheromone binding or complexing with antennal protein, then the change in absorbance should have rapidly reached a plateau that would have been dependent on the

concentration of the two reactants, antennal protein and pheromone.

To determine whether the reaction was unique to the antennae, particle-free supernatant fractions of haemolymph and legs were prepared and assayed for pheronome reactivity (Fig. 1*d*). The leg proteins were less active than the antennal proteins, and the haemolymph proteins were even less active. No measurable reaction occurred when pheromone was incubated with bovine serum albumin. However, preparations from the antennae of females were as reactive as the male preparations. A behaviourally inactive saturated analogue of the pheromone, dodecan-1-ol acetate, was assayed with the antennal, leg and haemolymph protein preparations. There was less reaction with dodecan-1-ol acetate in all three preparations than with the pheromone. These results indicate that the reactive protein showed some specificity for the pheromone and was primarily localized in the antennae.

To demonstrate more conclusively that interaction of the pheromone with the antennal supernatant was enzymatic, we monitored the rate of pheromone disappearance during the incubation with antennal proteins by gas chromatography[12]. Sonicated pheromone in distilled water (final concentration 2.02×10^{-2} M) was incubated with 2.0 ml. of antennal homogenate (100 mg wet weight ml.$^{-1}$), 0.2 ml. aliquots were collected during the reaction at 20° C and each aliquot was extracted with 0.5 ml. of anhydrous ether. One 0.2 ml. sample was held in a boiling water bath for 15 min before a 60 min incubation and then extracted. Gas chromatography of this sample gave one peak with a retention time identical to that of the pheromone. All other samples gave two peaks, one that represented the pheromone and a second one that had a retention time identical to that of (Z)-7-dodecen-1-ol, the alcohol moiety of the pheromone, which is a potent inhibitor of male attraction to the pheromone[12]. A time-dependent reduction in pheromone peak and a simultaneous increase in the height of the alcohol peak were observed. The ratios of the pheromone peak height to alcohol peak height values were 93.0, 47.5, 15.5, 8.7, 4.0, 2.2, 1.77, after 1, 5, 9, 15, 30, 45, and 60 min, respectively. These results demonstrated an enzymatic hydrolysis of the pheromone to the alcohol. In a subsequent experiment, the relative percentage of pheromone converted by the antennae, haemolymph, and legs was 33.9, 10.1 and 6.5% per 60 min.

When the inhibitor, (Z)-7-dodecen-1-ol (final concentration 2.5×10^{-3} M), was incubated with antennal supernatant (100 μg protein ml.$^{-1}$), it produced a rapid initial positive increase in absorbance which gradually declined to a negative value during the first 5 min, after which a plateau was reached that was stable for over 1 h (Fig. 1*e*). Addition of more inhibitor caused another, but slower, positive increase in absorbance change. This response, by contrast to that obtained with the pheromone (Fig. 1*e*), suggested that the absorbance change was due to reversible conformational transitions in proteins binding the alcohol as noted by M. Laskowski[13]. Incubation of the antennal preparation (100 μg protein) with the inhibitor (1.3×10^{-3} M) for 15 min before addition of the pheromone (1.3×10^{-3} M) did not prevent the absorbance change induced by the pheromone.

The significance of the *in vitro* conversion of the pheromone to inhibitor in the mechanism of olfaction by this insect remains obscure. There is no direct indication that this reaction is related to transduction[1]. It is possible, however, that enzymatic hydrolysis of the pheromone to the alcohol is a mechanism which regulates adaptation in the neurone and/or is a means of biologically inactivating the pheromone to prepare the dendritic receptor membrane for subsequent stimulation. This reaction may also be useful in obtaining inhibitors in other species whose mating behaviour is mediated through pheromones.

We thank L. L. Sower for the gas chromatographic analyses and J. H. Tumlinson for the (Z)-7-dodecen-1-ol.

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Received September 18; revised October 5, 1972.

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Morphogenesis and Regulation in Spite of Continued Mitotic Inhibition in *Xenopus* Embryos

There is evidence that the development of pre-patterns in vertebrate embryos involves not the counting of the cells but the erection of a "map" of information whereby zones of space are devoted to development of particular differentiations by cells within them. Thus in amphibian neurulae following initial removal of much of the totipotent blastula, abnormally few cells create a whole pattern, although each cell has normal dimensions. Conversely, in normal-sized *Xenopus* embryos having haploid cells, an appropriately larger-than-normal number of these smaller cells is assigned to each somite, the somite blocks being normal in number and dimensions[1].

Table 1 Inhibition of Cellular Multiplication during *Xenopus* Morphogenesis

Experiment	Cell number/embryo (without yolky endoderm) (s.e. in parentheses)				
	Controls at stage of operation	Control		Blocked at operation	
		Stage 14/15	Stage 22	Stage 14/15	Stage 22
I, Block with colcemid	4,300 (300) (stage 10+)	17,800 (500)	30,600 (400)	4,600 (300)	4,700 (300)
II, Block with mitomycin C and colcemid	5,700 (400) (stage 10½)	18,200 (400)	28,900 (500)	6,500 mitomycin (300) 5,600 colcemid (300)	6,200 mitomycin (300) 5,900 colcemid (300)

Cell numbers are the averaged results of counting four haemocytometer samples from each of two cell suspensions made with synchronous pairs of embryos at the morphological stages described. They are given, together with their standard errors, to the nearest 100 cells.

It has been suggested[2] that the cell cycle is necessary to normal development in other ways, either for the adjustment of cells' developmental tendencies in response to regulative changes in the pre-pattern of "map" or else as part of the ongoing process of cell commitment and histodifferentiation itself.

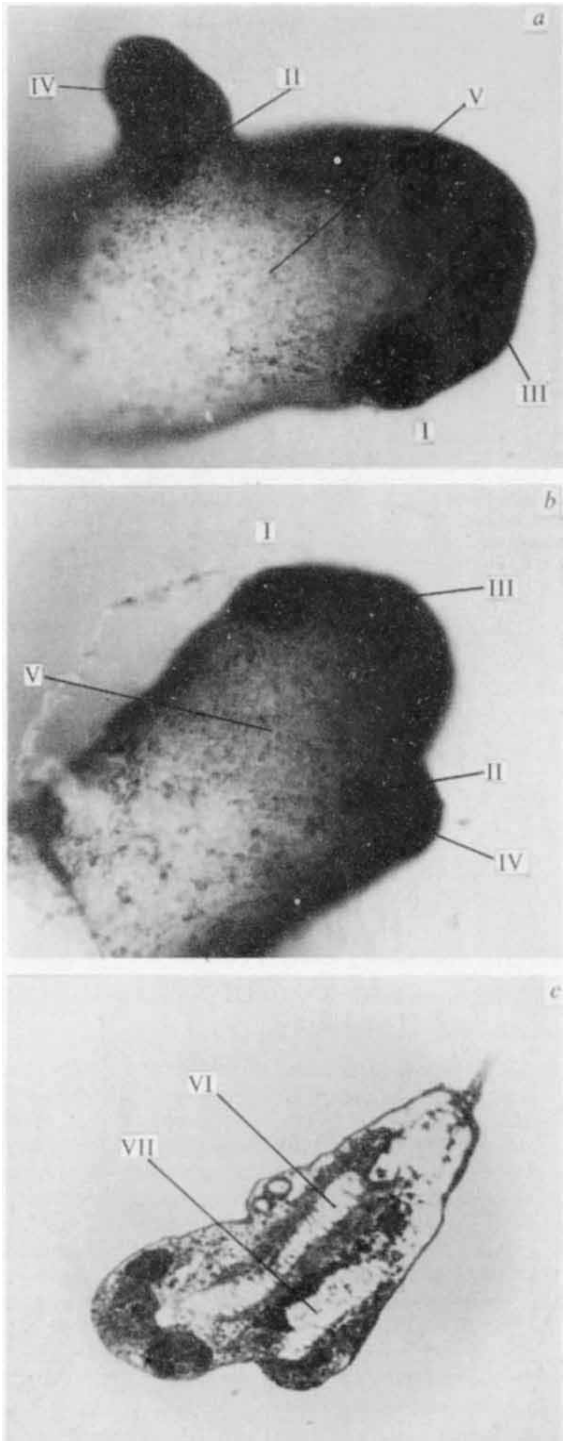

Fig. 1 *a* and *b*, Ventrolateral views of living embryos at about stage 25, following operations at stage 10 when additional head organizers were implanted. *a*, Control; *b*, cell cycle blocked with mitomycin C from time of operation; *c*, horizontal section of operated embryo at about stage 27, colcemid blocked, showing dual axial structure most readily seen in the paired, histodifferentiated notochord. I, Host cement glands (mouth precursor regions); II, cement glands of secondary inductions; III, forebrain fields of hosts; IV, forebrain fields of secondary inductions; V, equivalent regions, for visual comparison of average surface cell diameter; VI, host, and VII, secondary notochord, both showing histodifferentiation.

Recent work in an insect system[3] suggests that the first of these situations applies there.

Opportunity to test further the relation of cell number and an ongoing cell cycle to at least the early phases of amphibian differentiation has been provided by my finding that cell division in beginning gastrulae may be entirely inhibited, either with colcemid or with mitomycin C, and that this is followed by essentially normal development up to early tailbud stages. Cell counting has also shown that mitosis is arrested within a time, from application of the drugs, short relative to the normal cell cycle time in gastrulae. Gastrulation and neurulation movements are unaffected by mitomycin C, which is believed to cause intra-chromatid crosslinkage of DNA, preventing subsequent rounds of chromosomal replication without necessarily rendering chromosomes abnormal as RNA templates[4]. The movements are significantly slowed, although normal, under colcemid, a finding not surprising in view of the suspected involvement of microtubule systems in embryonic cell movements.

At stage 10^+ (ref. 5), the early gastrula subjected to mitotic inhibition in these experiments, almost all the territories of amphibian embryos are developmentally labile and pluripotent[6]. By late neurula stages cells are specialized in morphology and activity, and probably[7] in RNA transcriptional patterns, so the finding with colcemid has seemed startling in view of the condensed state of chromosomes as held in metaphase arrest. Histology shows, however, that in spite of permanent absence of mitoses, these nuclei return after some hours to an interphase-like configuration, having the nucleoli normal to postgastrular stages.

By late neurula stage 22, some 12 h after the blockage of mitosis, differentiation potency within the mesoderm and overlying, induced nervous system has become broadly restricted according to cell position[6]. The normal cell number at this stage is some 7 or 8 times that found in the early gastrula and in the inhibited embryos, representing some three cell generations. Thus the cell diameter difference of approximately 2 to 1 found along the dimensions of their structures in histological sections is as expected.

Table 1 shows results, in experiments on two separate batches of eggs, of cell counting following inhibition of cytokinesis. Details of handling of embryos, and of the operation mentioned below, are presented elsewhere[8]. Demembranated embryos were placed at stages 10^+ or $10\frac{1}{2}^+$ (ref. 5) on a 2% 'Agarose' bed in one-third strength Holtfreter solution (*p*H 7.2) containing either 0.05% colcemid (CIBA) or 40 γ ml.$^{-1}$ mitomycin C (Sigma, London). The blastocoel was gently flushed by micropipette, through a small rent made animally, ensuring access to all cells by the drug, After 45 min, with the rent fully healed, embryos were transferred to one-tenth strength Holtfreter, on glass, containing a holding concentration of either 0.015% colcemid or 20 γ ml.$^{-1}$ mitomycin C. Controls were similarly treated without exposure to antimitotic agents.

Pairs of embryos at the stages shown were placed in Ca^{2+} Mg^{2+} free Holtfreter with EDTA 150 mg l.$^{-1}$ (*p*H 8.2) for cell-counting. The large, yolky vegetal cells of early gastrulae, or their well known derivatives in later stages, were rapidly dissected free and discarded, as their variable size and fragility rendered accurate cell-counting difficult. Each pair of embryos was then transferred into 0.3 ml. of the same medium and gently pipetted to a single-cell suspension.

The results shown are typical of those in several further experiments. Cell division is effectively abolished in embryos blocked by both these agents. There is no evidence for breakaway from the block between stages 14–15 and stage 22, after which cell-counting by the direct method used is impossible because of histodifferentiation and matrix secretion. In each of six experiments employing direct cell-counting, however, no mitoses have been observed histologically in parallel blocked material at stages 26–27, some 4 h later, when notochord vacuolation, muscle-cell striation and spontaneous twitching have developed.

By performing a classical operation upon gastrulae, simultaneously blocking the cell cycle as described, I have tested the proposition that cells must traverse some part of the normal cell cycle, in order to respond appropriately to information assigning them to a changed position in a pre-pattern. An additional stage 10 dorsal blastoporal lip is implanted into a host early gastrula at a wide angle from its own organizer[8]. In such circumstances, a secondary anterior pattern of differentiation tendency and inductive activity is induced, leading by neurula stages to a double axial structure anteriorly, fusing to a single one posteriorly. Vital staining of implanted organizers shows that most of the new pattern extends over host cells.

Fig. 1 shows stage 25 embryos following such operations, with their dual anterior axial structure. Cell counting during this experiment showed a 7.5 : 1 cell-number ratio between control and blocked operated embryos by stage 22. The histological appearance of such a double set of axial structures, at stage 27 in a colcemid blocked embryo, is also shown. Counting data show that the cell cycle must be inhibited, following imposition of the drugs, well within the half hour taken by the graft to achieve normal cell contacts with host material.

Anatomical results of these operations are entirely similar over a series of some fifteen control, colcemid inhibited, and mitomycin C inhibited embryos in three experiments, where cell number appropriate to controls at time of operation is still observed at stage 22 among the operated embryos themselves.

Thus such differentiation as expressed in normal morphogenesis by tailbud stages, including the functional histodifferentiation seen in muscle and notochord, has developed in the absence of mitosis or of normal chromosomal replication. Further, such inhibited cells have altered their fate and final committed state appropriately upon becoming part of a new morphogenetic field. These results tell nothing, however, about the nature of the information constituting such fields[9].

I thank Rosi Tucker and Ann Blanshard for assistance, and the SRC for support.

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Received September 19; revised October 20, 1972.

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Ultrastructure of Synaptic Vesicle Formation in Cerebral Cortex

SYNAPTIC vesicles have assumed a role of singular importance in models of synaptic function as morphological evidence of their existence appeared simultaneously with the "quantal" theory of transmitter release[1]. The problem of synaptic vesicle origin, essential to understanding this role, has led to extensive investigations. Electron microscopic studies[2-4] have demonstrated synaptic vesicles attached to the axolemma or open to the extracellular space, and suggest that some synaptic vesicles form by a process of micropinocytosis at the presynaptic terminal membrane. Freeze-etched preparations, besides showing smaller micropits (synaptopores) at presynaptic sites, have shown similar plasmalemmal vesicles at non-synaptic sites[5]. A micropinocytotic origin of synaptic vesicles remains uncertain, however, and other workers suggest an intracytoplasmic mechanism of origin[6,7].

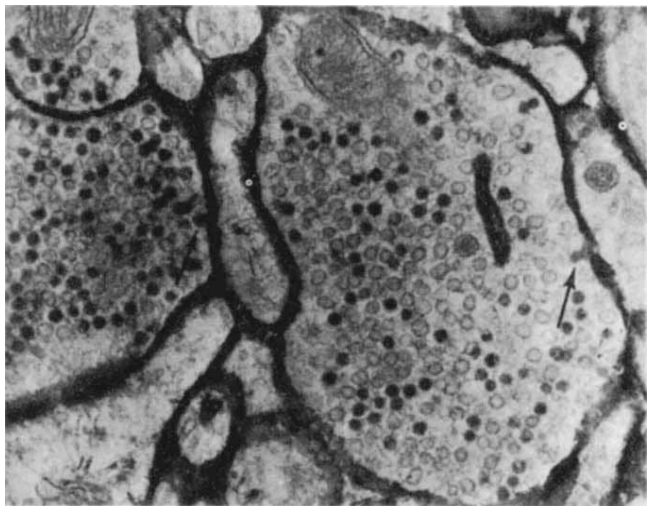

Fig. 1 Cortex extracellular space is filled with electron dense reaction product of horseradish peroxidase (HRP) 1 h after subarachnoid application. Presynaptic terminals show many of their synaptic vesicles filled with tracer and two vesicles (arrows) are intimately associated with the plasmalemma. ×53,000.

Electron dense protein tracer techniques make it possible to study communicating events between the extracellular space and intracellular structures and to bypass the brain barrier system[8]. Here we investigate the relationship between synaptic vesicles and the extracellular space in mammalian neocortex using horseradish peroxidase (HRP) applied directly as a tracer. We show electron microscopic evidence of remarkable labelling of synaptic vesicles and frequent loading of "coated" pits with peroxidase at the presynaptic terminal membrane.

Similar results were obtained from ten rabbits, two cats, and one monkey using either of two methods performed separately under halothane anaesthesia. In the first the subarachnoid space was exposed by a 10 mm craniectomy with a plastic ring attached to the surrounding bone creating a well to hold the HRP.

In the second a 27 gauge needle was inserted through the intact dura into the interhemispheric subarachnoid space for HRP injection. Approximately 20 mg of HRP (type II, specific activity, RZ 1.9 of possible RZ 3, Sigma Chemical Co., St Louis) in 0.5 ml. Ringer solution was injected within 30 min. The Ringer solution contained 147 mE Na, 4 mE K, 4.5 mE Ca, and 156 mE Cl (*p*H 5.2) and was allowed to reach room temperature (24° C) before injection. The injection technique was better for tissue preservation but HRP dispersion was less controllable. Tissue was fixed by intravascular perfusion of aldehyde fixative[9] at various times from 15 min to 4 h after HRP placement. Cortex treated in an identical manner, but without introducing HRP, served as a control. Tissue blocks were excised sharply and 50 μm sections cut with a 'Sorvall' tissue sectioner. Sections were incubated for 30 min in a solution containing hydrogen peroxide and 3,3′-diaminobenzidine[10], postfixed with osmium, and stained with uranyl acetate *en bloc*[11]. After routine dehydration and embedding, thin sections, unstained or stained with lead citrate, were studied on a 'Philips 300' electron microscope.

Within the area of HRP dispersion there was uniform flooding of the extracellular space with tracer. Almost all presynaptic terminals had labelled vesicles, many with as much as 80% of their vesicles containing HRP tracer (Figs. 1 and 2). These vesicles which were 300-500 Å in size, bounded by a trilaminar limiting membrane and dispersed among similar,

but electron lucent vesicles, appeared indistinguishable from synaptic vesicles. Tracer was contained within vesicles or occasional tubular structures (Fig. 1), but not free in the cytoplasm, indicating the vesicles were loaded prior to fixation[12]. A survey of micrographs revealed no tracer in sectioned axons.

Invaginations of various depths and configurations from shallow depressions to deeper bell-shaped and U-shaped profiles in the plasmalemma of presynaptic terminals are shown in Fig. 3. These "pits" were loaded with HRP tracer and, although not specifically stained to show a peripheral structure, they generally appeared to be coated (Figs. 2 and 3). They were apparently constricted at their base forming a spherical vesicle resting on a small cone of plasmalemma (Fig. 3*d*), and were able to separate, becoming interspersed among other vesicles in the terminal. Microinvaginations were appreciably more frequent at non-synaptic sites. Occasional microinvaginations (less than 2%) could be identified directly opposite the postsynaptic density but another group (approximately 20%) appeared to border the synaptic cleft and the remainder were found at non-synaptic sites. The sequence of images shown in Fig. 3 suggests that "coated" vesicles, previously described in presynaptic terminals[2,13], communicate with the extracellular space where they become filled with tracer during the process of micropinocytosis. It is possible that HRP-loaded "coated" vesicles may explain the many labelled synaptic vesicles in these experiments[13]. These observations could support the theory that some synaptic vesicles form by microinvagination of the plasmalemma.

Variability in the number of labelled vesicles among different terminals, both in time and location, was observed, but not yet quantified. Experiments at the neuromuscular junction indicated that resting synapses show little peroxidase uptake while stimulated preparations show many HRP containing vesicles[14,15]. No specific attempt at synaptic stimulation was made during our experiment, although the experimental conditions could have stimulated local increased synaptic activity to account for the labelling. In this case, the HRP present in terminals could be an indicator of synaptic activity during the observation period.

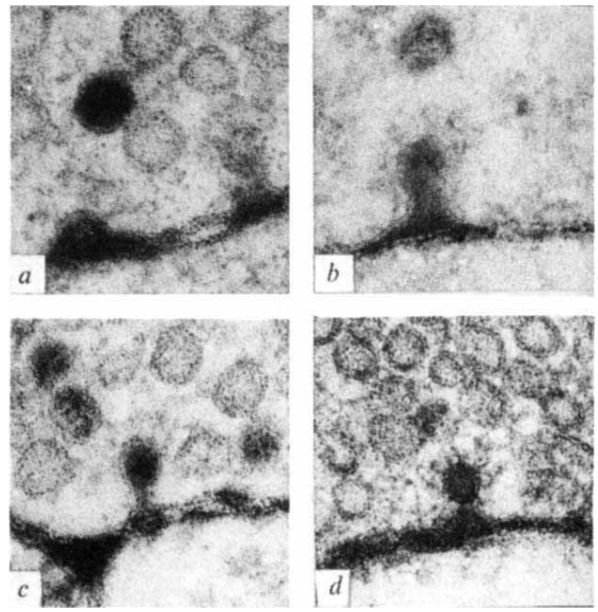

Fig. 3 Images consistent with micropinocytosis at presynaptic terminals from different animals. Four degrees of association shown between the "coated" pits and the plasmalemma. *a*, Tracer fills a shallow invagination in the plasmalemma of the presynaptic terminal. ×150,000. *b*, The membrane of this tracer filled "coated" pit is in continuity with the plasma membrane, allowing communication with the extracellular space through a large neck. ×165,000. *c*, The membrane connecting this "coated" vesicle and the plasmalemma has constricted, leaving only a thin channel between the loaded vesicle and the extracellular space. ×140,000. *d*, This HRP loaded "coated" vesicle appears resting on a cone of plasmalemma and may be in the process of losing contact with the axolemma. ×150,000.

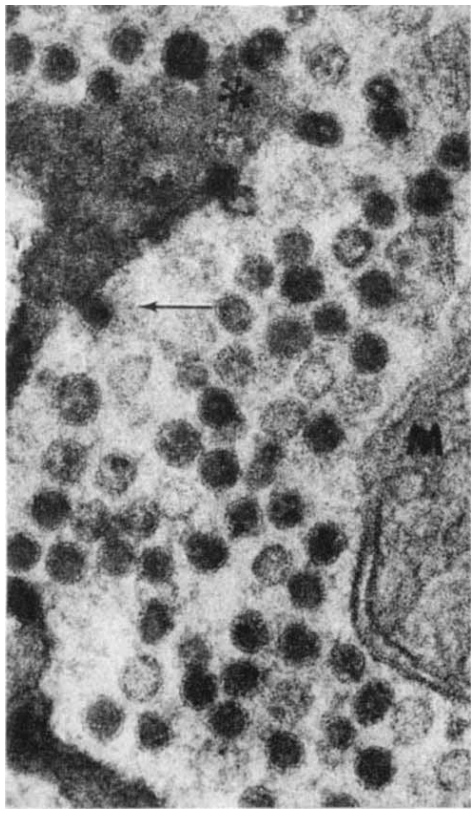

Fig. 2 An axon terminal containing many vesicles labelled with extracellular tracer. A mitochondrium (*m*) is present. Several labelled vesicles (*) are clustered near the tangentially sectioned plasmalemma and a "coated" vesicle (arrow) appears fused with the plasma membrane. ×106,000.

Our findings suggest that frequent communication between the extracellular space and synaptic vesicles occurs, and support the theory that this is accomplished through a mechanism of micropinocytosis by "coated" pits and vesicles. Whether there is any difference in direction (that is, discharging or loading) between invaginations adjacent to the synaptic cleft or elsewhere on the terminal remains unknown and is a limitation of this cytochemical tracer technique. Endocytosis appears to be an important property of presynaptic terminal surface membrane as well as surface membrane of the perikaryon[16], although the controlling conditions are as yet unknown. Endocytosis may play an important role in synaptic function; but this may or may not be immediately related to synaptic transmission[17].

This work was supported by grants from the US Public Health Service.

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Received August 18; revised November 20, 1972.

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Morphine Withdrawal Syndrome Responses to Cholinergic Antagonists and to a Partial Cholinergic Agonist

MORPHINE impairs the release of acetylcholine (ACh) at muscarinic and nicotinic sites in the periphery[1-3], and within the brain[4-8]. Paton[9] suggested that morphine's ability to impair ACh release might be the origin of the morphine withdrawal reaction. He proposed that ACh could accumulate within cholinergic terminals during habituation to the narcotic and then flood out onto cholinoceptive tissues when the drug is withdrawn, giving rise to opiate withdrawal symptoms. He later[10] outlined the autonomic imbalance seen during narcotic abstinence, and suggested that supersensitivity might develop in cholinergic receptors during morphine habituation, as a result of the deprivation due to impaired release.

We have attempted to test the cholinergic hypothesis by administering drugs which act specifically on cholinoceptors to rats undergoing withdrawal from morphine. In one study seventeen male Sprague-Dawley rats (initially 100 g) were given morphine sulphate by i.p. injection twice daily; doses were increased regularly from 20 mg kg^{-1} per day up to 600 mg kg^{-1} per day over 21 days. The animals responded with a severe abstinence syndrome when the drug was withheld. Severity of the total withdrawal syndrome was assessed by scoring and then averaging the prominence of various behavioural signs of withdrawal upset (Fig. 1). A "blind" design was used; drugs, solutions and animals were all coded until after the study had been completed and the results analysed. Rats were divided into three groups during withdrawal and given i.p. injections of either saline (control group—5 rats) or one of two anticholinergic treatments (mecamylamine group—6 rats; atropine-mecamylamine combination group—6 rats) at various times during the withdrawal reaction (Fig. 1). Significance of the data was assessed by Student's *t*-test for unpaired data using the square-root scale transformation[11] to check the validity of inferences made from data consisting of small whole numbers with many zero results.

In the first check period after initial withdrawal from morphine (14–15 h) there were no significant differences in withdrawal severity between any of the three groups (Fig. 1). Withdrawal severity rose significantly in both groups of drug-treated rats after injection of the anticholinergic drugs at 22 h but the increase in the saline-treated group was not statistically significant. The atropine-mecamylamine mixture, however, reduced withdrawal severity when administered at 42 h after initial withdrawal; this may be seen by comparing scores for the saline- and mixture-treated animals during the 44–45 h check period. Withdrawal severity in both drug-treated groups but not controls was significantly less during the 49–50 h check, which followed injections at 48 h, than during the 44–45 h check. The effects of the 48 h injection seemed to wear off after 10 h, scores for drug-treated animals at 58–59 h being similar to controls in the same check period but consistently higher than scores for the drug-treated animals during the 49–50 h check.

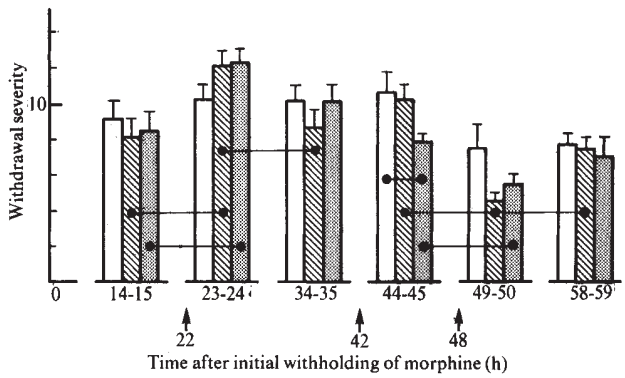

Fig. 1 Effects of anticholinergic drugs on severity of withdrawal from morphine. The narcotic was withheld from 17 rats tolerant to high doses of morphine sulphate (see text) by substituting saline injections for the regular morphine injection in all the rats at 0 h. Withdrawal severity was assessed by scoring, on a discrete scale of from 0–5 points, the following behavioural signs: piloerection; shrieking and escaping upon gentle handling; shrieking and attacking upon being poked with a needle; startle response to a puff of air on neck or haunches; hunch-backed posture; territorial exploring; apparently non-purposive motor hyperactivity ("restlessness"); muscle twitches; body tremor. Severity of the total withdrawal syndrome was quantitated by finding the average total score of all signs per animal for each group of rats over any given check period. Such averages are represented by heights of the vertical bars. Open bars represent withdrawal severity for rats which received saline injections (controls, 5 rats); hatched bars represent that for treatment with mecamylamine hydrochloride 6.0 mg kg^{-1} (6 rats); shaded bars that for treatment with a combination of mecamylamine hydrochloride 6.0 mg kg^{-1} with atropine sulphate 4.0 mg kg^{-1} (6 rats). Drugs were injected at times indicated under arrows. Vertical lines represent standard errors; horizontal lines joining bars indicate significant ($P < 0.05$) differences between the scores.

There thus appears to be a biphasic response to anticholinergic treatment during withdrawal from morphine habituation. Collier, Francis and Schneider[12] have described similar results with atropine, chlorophenylalanine and indomethacin in morphine withdrawal precipitated by naloxone in rats. They observed, in individual signs of withdrawal upset, biphasic responses to all three agents and concluded that acetylcholine, 5-hydroxytryptamine and prostaglandin(s) are all involved in morphine withdrawal. A biphasic or even multiphasic response to anticholinergic treatment is predictable within a cholinergic mechanism, however, because it is unlikely that all synapses will recover at the same rate or be at the same stage of derangement at any given time during the withdrawal reaction. Adding a cholinergic antagonist early in the abstinence syndrome might therefore actually increase the deprivation of cholinergic activity at those synapses not yet effectively relieved of their morphine burden whilst, at fully-recovered synapses, not causing sufficient blockade to overcome excessive cholinergic stimulation.

Our own results suggested that withdrawal severity might be reduced throughout the entire syndrome by the administration of a drug which could prevent development of receptor supersensitivity during impairment of release but which could also antagonize cholinergic drive during periods of excess[28]. These criteria might be met by a partial cholinergic agonist. Choline chloride is a weak cholinergic agonist[13-15] which readily enters cells and passes the blood-brain barrier in spite of its quaternary nature[16-18]. An initial study on six morphine-habituated rats tested the effects of choline on withdrawal from morphine addiction. The effect of choline chloride on the severity of withdrawal is shown in Fig. 2. The treatment clearly diminished intensity of the withdrawal syndrome monitored over a 73 h period in comparison with the control addicted animals which were given saline injections only during withdrawal over the same period. Choline-treated animals suffered less weight loss, maintained normal grooming

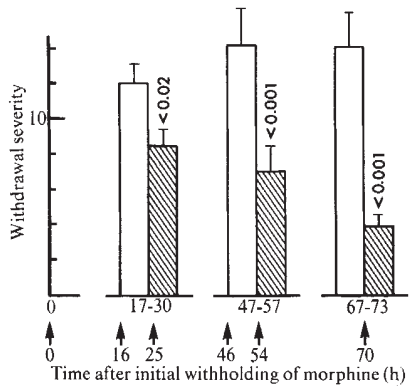

Fig. 2 Effects of choline chloride on severity of withdrawal from morphine. The narcotic was withheld from 6 rats tolerant to high doses of morphine (see text). Either choline chloride 100 mg kg^{-1} (choline-treated group, 3 rats) or saline (control group, 3 rats) was substituted for the regular dose of morphine at 0 h. Withdrawal severity was assessed as described in caption to Fig. 1 except that additional scores were assigned for signs of salivation, erection and ejaculation while a negative score was assigned for grooming. Values for severity in the period 17–30 h are the mean of that observed in four individual check periods over that time; there were three individual check periods in each of the time periods 47–57 h and 67–73 h. The rats in both groups were reinjected with their initially-administered solutions at times indicated under arrows. Open bars: withdrawal severity in rats treated with saline injections during withdrawal (controls); shaded bars: that for rats treated with choline chloride. Vertical lines represent standard errors, significance of differences between scores for saline-treated and choline-treated groups indicated by *P* values above shaded bars.

and appeared in general to be normal healthy rats as compared with the saline-treated controls. The ameliorating effect of choline on withdrawal severity was not biphasic, by contrast with the results described here for cholinergic antagonists or for drugs interfering with other putative neurotransmitters[12]. Choline-treated rats showed less severity than controls in all ten of the individual check periods from which the data summarized in Fig. 2 were drawn.

Involvement of neurotransmitters other than ACh in the morphine withdrawal syndrome has been considered[12,19–22] but there is controversy over their importance[23–27]. Choline chloride, regardless of the possible involvement of non-cholinergic systems, appears to be very effective in treating the narcotic withdrawal syndrome in rats throughout a long period of monitoring. We explain this as due to a major involvement of a cholinergic mechanism in this syndrome and are seeking an optimal treatment in further studies on cholinergic systems altered by chronic administration of morphine.

We thank Mr John Davidson Brown for technical assistance, Dr Deepak Bose and Mrs Marge Turner for assistance with coding of drugs and Dr Ian R. Innes for gifts of animals and drugs. This work was supported by the Medical Research Council of Canada.

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Comments on the "Isolation, Identification and Synthesis of a Specific-behaviour-inducing Brain Peptide"

DURING the past six years a remarkable series of publications has appeared, claiming the transfer of various specific learned behaviours from animal to animal by i.p. injection of extracts made from brains of trained donors. These are cited in the latest paper[1], in which Ungar and his colleagues propose an amino acid sequence for a pentadecapeptide isolated from the brains of donor rats trained to avoid a dark box. Injection of this material into untrained recipient mice is alleged to transfer the learned dark avoidance. The molecule has been named scotophobin, after the Greek words meaning "fear of the dark".

The long controversy over the claims for transfer of learned behaviours was reviewed by W. W. Stewart[2] in an article to which Ungar *et al.* replied[3]. Unfortunately, the Stewart review dealt very largely with the weakness of the claims for a particular amino acid sequence, while paying far too little attention to more crucial weaknesses at the heart of the interpretation. After all, some oligopeptide material has evidently been isolated from trained donor rats. If this material—whatever its exact structure or state of purity—is truly capable of specifically transferring a learned behaviour to untrained recipient animals, the discovery certainly ranks among the most fundamental in modern biology. It is because of the far-reaching implications that so much controversy has been aroused. For the same reason, it is of the utmost importance that the experimental

evidence be as complete and convincing as possible. I shall point out two major uncertainties about this work, which apply as surely to the present experiments with a peptide as to previous experiments with crude brain extracts.

The first question is: Can the investigators state precisely the conditions for carrying out an assay, in such detail that competent scientists elsewhere can reproduce their results? Our own repeated failure[4] could be written off as the bungling work of incompetents, were it not matched by published experiences of some others, as cited by Stewart[2] and by Ungar[3,4]. It would be interesting to find out how many tried and failed but did not feel such an outcome was worth publishing. Is Ungar's dictum, "Negative results are always easier to obtain than positive ones,"[4] really true? It has a strange ring to me, as a pharmacologist. Suppose we are told that the median analgesic dose of a new narcotic drug in a given strain of mice is 1 mg kg^{-1}, using the hot plate procedure with specified parameters of time and temperature. If the information is correct, it will certainly be harder, not easier, to find no analgesic activity at all, at sufficient doses. The truth is, by following the specified procedures, we can expect to confirm that the drug is an analgesic. One of the most disconcerting things that can happen to a scientist is the failure of other investigators to confirm his findings. Clearly, something is wrong, at the very least a failure to specify all the relevant conditions of the experiment. Such a situation calls for the most intensive effort to resolve the difficulty, and thereby to sharpen understanding of the relevant variables. It is hardly reasonable to find fault with others for not exploring all the variables that should have been worked out in the first place. It is universally agreed that reproducibility is a necessary condition for accepting the validity of a scientific finding.

The second question goes to the heart of the interpretations placed by Ungar upon his work. Let us accept, for the sake of argument, that the results are too difficult to obtain except in the expert hands of a few, but that they are none the less real. What then, has been shown, and what does it mean? To answer this, and to understand the crucial defects in Ungar's experimental design, one has to have seen or done these experiments oneself. In discussing "scotophobin", I shall discuss only those experiments employing dark avoidance, as this was the paradigm used in the isolation of the oligopeptide.

In Ungar's procedure[4] a donor rat is placed in a centrally placed, illuminated white box, which is connected by tunnels to another illuminated white box on one side, and to a dark box on the other. The rat follows a normal instinctive behaviour; it runs into the dark box. There it is given foot shock, and it runs out again. This animal, it should be noted, will rarely enter the dark box again spontaneously. Indeed, this is an example of the classical paradigm known as "one-trial learning", which has been used extensively by McGaugh[6] and others to study short- and long-term memory. If learning to avoid the dark box led to the production of a "memory transfer factor", it should be possible to prepare active extracts after such a single trial, in which a long-lasting aversive conditioning (dark avoidance) is established—if not immediately, then surely at some time later, when the memory has been consolidated. But Ungar knows and admits that this will not work. "In passive avoidance," he writes, "training is completed on the first day, but several more days are required for the brain to accumulate the excess of material necessary for transfer."[4]. This means several more days of training, not merely several days to accumulate the "excess of material necessary for transfer". In other words, there is an utter lack of temporal relationship between learning and consolidating the dark avoidance, on the one hand, and the appearance of the alleged transfer material, on the other.

What actually happens in the training of donor rats is this. After the animal is shocked and runs out of the dark box, the investigator has to sieze it, and force it through the tunnel into the dark box, where it is again shocked and runs out. This extraordinary stressful procedure is repeated five times in rapid succession, to complete the first day's training session. Ungar's description is: "If he does not run back (this is the rule after the second session), he is pushed through the gate into D and shocked. Each daily session consists of 5 shock periods of 5 s each, separated by intervals of 10 s"[4].

The next day, when the rat is first placed in the white box, it immediately shows fear. It cowers in a corner, defaecates, trembles, and often tries to leap out of the box. Again the investigator must seize the animal, which struggles vigorously and tries to bite, until it is pushed and twisted through the tunnel, locked into the dark box, then shocked and allowed to run out. Sometimes the rat behaves in a confused manner, "freezing" inside the dark box, and refusing to run out; then the investigator has to push it back through the tunnel into the white box. This whole procedure (five trials in succession) is repeated on six successive days. Then the animal is decapitated. Thus, donor rats receive massive stressful manipulation as well as shock at an intensity and for a duration far greater than required to learn dark avoidance.

If a naïve observer were to watch the training procedure without being told that dark avoidance was the objective, and if he were then told that brain extracts from such animals induced a behaviour different from that induced by brain extracts of untreated controls, he would certainly conclude that extreme stress was the causative agent. Any competent scientist would know that the proper control group should consist of rats that received equivalent foot shock and intensely stressful manipulation, without the dark box contingency, that is, with nothing to learn. But one looks in vain for data on even one such adequate control experiment in Ungar's work. The only published controls are found in a table[7], where a total of twenty donor rats were subjected to shock in a lighted chamber or white box. No further description of the experimental conditions is offered, and there is no intimation that these animals were subjected to stressful manipulations. Other than this, and despite the publication of at least seventeen articles on the subject, one finds only undocumented assertions about the specificity of transfer of dark avoidance, or very limited data on the specificity of transfer of behaviours other than dark avoidance.

During my visit to Houston, where I observed Ungar's techniques at first hand, I noted that although he was recording latency (that is, time required for a recipient mouse to first enter the dark box), all his published data concerned dark box time (that is, total time spent in the dark box in a 180 s trial). I thought this curious, because if dark avoidance behaviour were really induced by the injections, the latency should be increased. This is elementary logic. Indeed, latency is the common and accepted measure for such behavioural phenomena among experimental psychologists. Yet Ungar has never used latency, and has never claimed to have shown differences in it.

Dark box time, on the other hand, which is Ungar's standard measure, would probably be sensitive to other behavioural effects. A recipient mouse that wanders about more because it is hyperactive would naturally be more likely to leave the dark box than a more passive animal. Amphetamine, for example, might well reduce dark box time. A recipient mouse whose perception or interpretation of the environment had been obtunded would be more likely to leave the dark box than an alert animal. Morphine, for example, might well produce this effect.

Investigators who were seeking alternative explanations to their own preconceived ones would have performed some

of the elementary controls suggested by the remarks above. My own guess is that if there is a real effect at all, and if there is a natural or synthetic peptide producing decreased dark box time (not "dark avoidance"), it is probably a non-specific factor induced by stress. This, in itself, could be a discovery of some importance. But it serves no useful purpose to claim the isolation and synthesis of "the coded molecule involved in dark avoidance"[4] until three things have been done:

(1) The precise conditions for the transfer of "dark avoidance behaviour" by crude extracts should be written down in detail and followed by several investigators, to ascertain once and for all if the phenomenon meets the test of reproducibility.

(2) Crude brain extracts from stressed control donors should be shown to lack the capacity to induce dark avoidance. This experiment must, of course, be carried out in a blind design, concurrently with extracts from a trained donor group. Ideally, the extracts should be sent (after coding) to several groups of investigators for evaluation, as one might do in the preclinical testing of a new drug.

(3) The oligopeptide should be shown to be present in brains of trained donors and absent from brains of stressed untrained donors. The work-up of material from both sources should be concurrent, each batch of material should be coded and unknown to those engaged in the isolation, and several groups should carry out the procedure. Since more than 4,000 rats were required for the isolation of "scotophobin", it may suffice, as a first step, to show that the specified fractionation procedures that led to the isolation of the peptide will produce biologically active fractions with trained donor brains but not with stressed control brains.

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Received August 25; revised November 9, 1972.

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Ouabain Binding to the Sodium Pump

IN the course of studying ATP hydrolysis by the sodium pump, we have measured ouabain binding to ox brain microsomes. Our results on binding in the presence of Na, ATP and Mg agree with earlier findings, but our results with Mg alone lead us to question the validity of models which have been proposed for the mechanism of the sodium pump[1-3].

To ensure that equilibrium was reached between bound and free ouabain, the time course of binding was measured at a ouabain concentration of 5×10^{-8} M (see legend to Table 1). With either Na + ATP + Mg or Mg alone, a plateau was reached after about 45 min, and 60 min was kept as the incubation time in all further experiments. The level of ouabain binding at equilibrium was the same with 5 mM $MgCl_2$, whether or not Na + ATP were added. A more stringent test was to determine the affinity (dissociation constant) and number of receptor sites for the reversible reaction

$$\text{enzyme.ouabain} \rightleftharpoons \text{enzyme} + \text{ouabain}$$

On raising the ouabain concentration (up to 2×10^{-7} M), there was more binding, of a kind that allowed a Scatchard[4] analysis of the results. The value for the dissociation constant with Na + ATP + Mg (Table 1) agrees with previous work[5], as does the turnover number, calculated from the number of binding sites. Table 1 shows, however, that the values for the dissociation constant and number of binding sites with Mg alone were not significantly different from those with Na + ATP + Mg. This result shows that the binding is quantitatively equivalent in the presence of either Na + ATP + Mg or Mg alone. Potassium inhibited the binding in both conditions equally, 10 mM being sufficient to reduce the binding to the low levels found in controls, to which no additions of Na, ATP or Mg were made. The binding reactions are not identical, however, because 100 mM sodium, the concentration employed in the Na + ATP + Mg condition, completely inhibited the binding with Mg. In previous work the binding with Mg alone has been stimulated by P_i, but there is conflict on whether the level reached is greater[2] than or less[1] than that with Na + ATP + Mg. We find no effect of P_i (up to 10 mM) in the presence of Na + ATP + Mg, whereas with Mg alone it caused a small, though significant, stimulation (25%).

One feature of the present study is that 2 mM EGTA was added to all solutions in order to remove traces of adventitious calcium. Tests with and without EGTA were made of ATPase activity and binding. The results showed the same degree of binding although there was a three-fold increase in ATP hydrolysis (see Table 1).

The results lead to three conclusions. First, calcium does not influence ouabain binding at the very low concentrations which inhibit ATP hydrolysis. There is therefore no obligatory correlation between the rate of ATP hydrolysis and the magnitude of ouabain binding. The second conclusion follows; namely, the ouabain binding site is spatially separated from the site at which calcium acts. This is consistent with inhibition by calcium from inside cells and by ouabain from outside. The third conclusion is that Mg alone is sufficient to cause a conformational change such that ouabain can bind to the same extent as with Na + ATP + Mg. This simple requirement is not a feature of models which have been proposed relating ouabain binding to the conformational state of the pump[1-3]. Before ouabain can become bound, it is clear that a conformational change must take place. This has hitherto been held to occur following the formation of either a phosphorylated intermediate or an enzyme-substrate complex. Our results show that ouabain can become bound in conditions in which neither of these two reactions could have taken place. It

Table 1 Ouabain Binding to Ox Brain Microsomes

Additions (mM)			Dissociation constant ($M \times 10^{-8}$)	No. of binding sites (per mg protein $\times 10^{13}$)	No. of experiments
Na	ATP	Mg			
100	3	5	2.35 ± 1.38	4.1 ± 0.4	6
None	None	5	2.61 ± 0.77	4.1 ± 0.6	4

Ox brain microsomes, prepared and assayed as described previously[6], contained sodium-dependent ATPase activity (5.3 μmol P_i liberated/mg protein per h), which was raised three-fold (to 15.7) when 2 mM EGTA was added. Ouabain binding was measured in a medium containing 2 mM EGTA, 20 mM imidazole-HCl (*p*H 7.6) and, as appropriate, 100 mM NaCl, 3 mM ATP, 5 mM $MgCl_2$ and ^{3}H-ouabain (NEN, New Boston, Massachusetts). After incubation at 37° C, small portions were cooled to 0° C and then centrifuged at 100,000*g* for 30 min. The pellets were resuspended in 5% aqueous 'Triton X-100', and the bound ouabain was assayed by liquid scintillation counting. The results are expressed as the mean ± standard deviation.

therefore remains to be proved which of the conformational states revealed by ouabain binding is directly related to the normal function of the pump.

We thank the MRC for financial support.

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Received October 13, 1972.

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A New Hypothalamic Pathway to the Median Eminence Containing Neurophysin and its Hypertrophy in Sheep with Natural Scrapie

THE hypothalamus is linked to the pituitary by two anatomically distinct neuronal neurosecretory pathways. The first, the hypothalamo-distal neurohypophysial system, commonly termed the supraoptico-neurohypophysial system (SNHS), arises in the "magno-cellular" cell bodies of the supraoptic nuclei (SON) and the paraventricular nuclei (PVN), whence their large axons descend via the internal infundibular zone to the posterior pituitary (Fig. 1)[1,2]. This system is characterized immunochemically by the neurophysins, the specific carrier-proteins for vasopressin and oxytocin[3–6].

The second, the hypothalamo-proximal neurohypophysial system, originates in "parvicellular" cell bodies in the hypophysiotropic area (HTA) of the basal medial hypothalamus and its fine axons terminate in the external infundibular zone of the median eminence and pituitary stalk (Fig. 1*b*)[2,7–9]. This pathway, containing many catecholaminergic neurones[10,11], conveys the specific adenohypophysial hormone-releasing factors[12,13], which have no known specific carrier-protein[14].

We have evidence for another neurophysin-containing pathway arising in the SON and PVN, but proceeding only to the proximal neurohypophysis and terminating in the external infundibular zone of the median eminence-stalk. We have found that a neurophysin-like antigen associated with pressor activity is normally present in the external zone of the infundibulum in sheep in amounts and in a form very similar to those found in the adjoining internal zone of large longitudinal axons of the SNHS.

Many clinical signs of natural scrapie disease of sheep are compatible with a progressive and severe disturbance of hypothalamic and pituitary function[15,16]. Severe losses of neurones in the SON and PVN are associated with degenerative lesions in the neurohypophysis[17], and the loss of neurones in the SNHS was considered to offer an adequate explanation for the clinical disturbances of water and electrolyte intake[17].

Livett and Parry[6,18] used a cross-species reactive neurophysin antiserum[19] to demonstrate the precise cellular location of neurophysin (or a substance so similar antigenically as to be cross-reactive) in the SNHS of normal and scrapie sheep and have correlated these semi-quantitative observations of neurophysin content with vasopressin estimations using the method of Dekanski[20]. Their results confirm the histological findings of Beck, Daniel and Parry[17], and provide evidence of degeneration of the SNHS with severe vasopressin insufficiency in the later stages of natural scrapie.

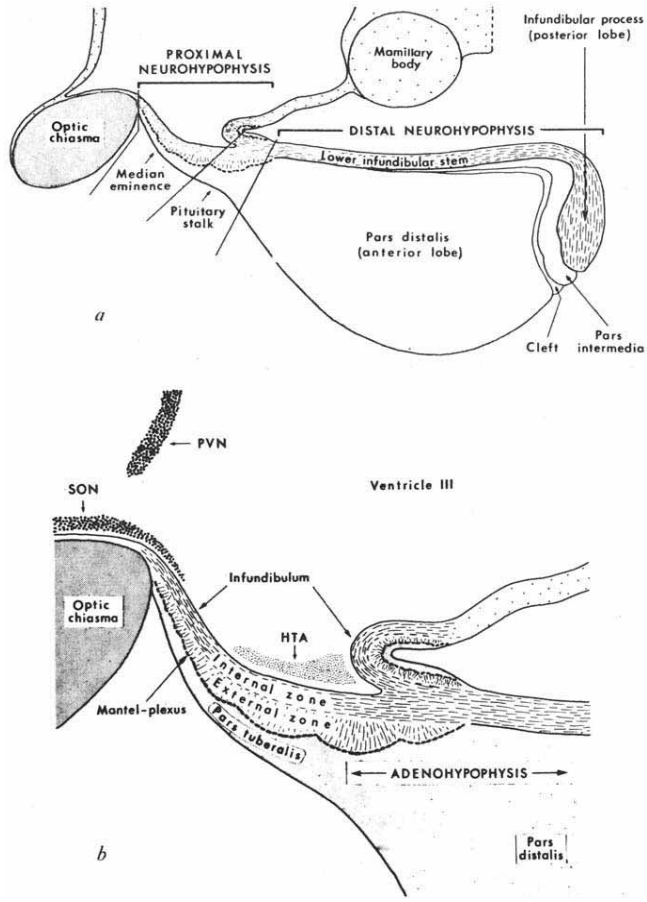

Fig. 1 *a*, Diagram showing the main components of the pituitary region in the sheep, as seen in the mid-sagittal plane. *b*, Part of *a* enlarged to show in greater detail the structure of the proximal neurohypophysis. Although this diagram represents a midline section through the cavity of the third ventricle, it indicates the approximate sites within the hypothalamus of the supraoptic nucleus (SON), the paraventricular nucleus (PVN) and the hypophysiotropic area (HTA).

On the other hand, the neurosecretory system sustaining what is presumably a neurophysin-vasopressin component in the external infundibular zone of the median eminence-stalk is preserved and is hypertrophic in scrapie sheep, showing a substantial increase of neurophysin immunofluorescence accompanied by an estimated two-fold increase in vasopressin content. The neurophysin fluorescence is particularly intense in the outer part of the external zone, where there is in the sheep an extremely dense network of short capillary loops[21], and also around the long portal vessels of the pars tuberalis, where it occurs frequently in the walls of the portal sinusoids. In the hypothalamus, however, no neurophysin immunofluorescence was observed except in the SON, PVN and in the intrahypothalamic tract originating from these nuclei.

Several considerations arise from these observations.

(1) We are not aware that neurophysin (or vasopressin) has been demonstrated previously in the external zone of the infundibulum[22,23], where the Gomori reaction for neurosecretion is usually negative in normal mammals[2], except the horse[24]. The external infundibular zone comprises the nerve terminals of the tubero-infundibular tract[2], the majority of which arise in the arcuate nucleus and contain dopamine[10,11], but with some containing noradrenaline[25] and some 5-hydroxytryptamine[26,27], while terminals of small axons from the SNHS occur in some mammals[28,29] and in birds[30].

(2) As specific neurophysin immunofluorescence occurred only in the SON and PVN neurones, we consider that there is in the sheep a tract from these nuclei running with the main tract of the SNHS caudally as far as the anterior (rostral) lip

of the entrance area of the infundibulum where it sweeps ventrally and outwards to terminate in the external infundibular zone and the Mantel-plexus, thus constituting a supra-optico-infundibular tract.

(3) The neurone populations of the SON and PVN are not anatomically and physiologically homogeneous, although characterized by intense specific neurophysin immunofluorescence.

(i) While the SON cell bodies are mostly magnocellular (MC), in the PVN there are a proportion, probably 25–30%, of parvicellular (PC) cells interspersed amongst the MC cells in the rat[31] and sheep[32–34].

(ii) After permanent interruption of the tract from these nuclei to the infundibular process at the level of the lower infundibular stem, e.g. as in posterior pituitary lobectomy, 40–50% of all SON and 33% of all PVN neurones survive[35]. If the interruption is more proximal in the mid-pituitary stalk, for example as in low stalk section and intrasellar hypophysectomy, the loss of neuronal cell bodies is greater, and only 25–30% of the MC cells of both nuclei survive in the dog[36] and rat[31], while the PC cells of the PVN remain unaffected[31].

(iii) When the level of axon interruption is more rostral and anterior to the stalk in the median eminence–infundibular area of the ventro-medial hypothalamus, the loss of neurones is increased to 80–85%, especially in the PVN, in the dog[36], the goat[37] and man[38], suggesting that a further leash of axons, with cell bodies mainly in the PVN, has been severed.

(iv) After axon interruption at levels (ii) and (iii) new "miniature" neural lobes form from the proximal stump by outgrowth of new nerve fibres, which become filled with neurosecretory material[9,39,40]. The more rostral the stump the smaller is the new neural lobe[35,41].

(v) Two fibre pathways from the PVN to the neurohypophysis are recognized[42]: (*a*) a rostral tractus paraventricularis cinereus[43,44] joining the main supraoptico-neurohypophysial tract to the posterior pituitary; and (*b*) a more caudal and medial direct paraventriculo-hypophysial (infundibular) tract in the dog[45], the rat[31] and the rabbit[46] intermingling with the main tract in the caudal median eminence–infundibular entrance area and probably terminating in the outer layers of the median eminence-stalk[47,48]. The rostral pathway is thought to control vasopressin release, with the caudal one concerned with oxytocin release[49].

(vi) When recording from single neurones of the PVN following antidromic stimulation from the neural lobe, Cross, Novin and Sundsten[50] found that only about half the PVN neurones recorded could be activated from the neural lobe and concluded that "a large portion of PVN neurones may not project to the neural lobe".

There is thus considerable evidence for neurone populations in the PVN, and possibly also in the SON, which do not project to the posterior pituitary, and are available for a supraoptico-infundibular neurophysin pathway, arising mainly in the PVN and proceeding via the medial paraventriculo-infundibular tract of Laqueur to terminate in the external zone of the infundibulum.

(4) The neurosecretory axons innervating the median eminence may therefore consist of at least two subsystems, with the supraoptico-infundibular subsystem distinct from the main tubero-infundibular subsystem. Ultrastructural studies of the median eminence of the rat[48], the mouse[51] and mammals[52], and the toad[53], show a variety of nerve fibres and axon terminals containing at least three types of granules as well as vesicles, compatible with the neuronal and functional heterogeneity one might expect with two subsystems.

(5) The increased neurosecretory material in the external infundibular zone of scrapie-affected sheep[6] probably lies within this supraoptico-infundibular subsystem. After bilateral adrenalectomy[54–57] and hypophysectomy[58], similar neurosecretory material also accumulates in the external zone, often in new fibre outgrowths from the internal zone[54], with an increased content of corticotrophin-releasing factor (CRF) in the median eminence-stalk[59]. This accumulation may be reduced markedly by cortisone therapy[60], suggesting that the amount of stainable Gomori-positive neurosecretory material in the external infundibular zone and its CRF content reflect adrenal function.

(6) The role of the vasopressin-neurophysin complex in the external zone of the infundibulum is unknown, but its presence could provide a basis for the view that vasopressin is an adreno-hypophysial hormone-releasing factor, especially for adrenocorticotrophic hormone (ACTH)[14,60–62], and possibly concerned with the quick systemic component of ACTH release[52,63,64].

(7) In natural scrapie the supraoptico-infundibular neurosecretory subsystem is preserved and undergoes a functional hypertrophy in the terminal stages of the disease, probably related to the progressive loss of the principal vasopressin-oxytocin neurosecretory pathway to the posterior pituitary, and to the disturbances of adrenal function[16].

Natural scrapie in our sheep population behaves as an hereditary disease controlled by an autosomal Mendelian recessive gene with full penetrance[16,65–67]. As we now have sheep populations of the three scrapie genotypes[66], the discovery of the neurophysin-vasopressin pathway to the proximal neurohypophysis and of the divergent action of the scrapie genome on this pathway compared with that to the distal neurohypophysis offers another model for hypothalamo-neurohypophysial studies.

This work was supported by grants from the MRC to Dr D. B. Hope, and from the National Genetics Foundation of New York to H. B. P. B. G. L. held a Nuffield Dominions Demonstratorship (Australia). We thank Dr L. O. Uttenthal for purified porcine neurophysin-II and Miss Wendy Jones for the vasopressin bioassay. The help of Dr M. M. J. Prichard has been invaluable.

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Received September 9; revised October 16, 1972.

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Physicochemical Aspects of the Action of General Anaesthetics

THE physicochemical aspects of the action of anaesthetics have been studied for many years, with general agreement on the following conclusions. No chemical reaction seems to take place as in the extreme case of rare gases anaesthesia can be realized under proper conditions[1]. Observations favour the membrane as the site of anaesthetic action[2], and the anaesthetic receptor seems to be of finite size and the anaesthetic agents molecules of limited size. It has been suggested that narcosis occurs when a critical fraction of space in the membrane is occupied by the volatile anaesthetic agent and it is generally agreed that narcosis is due to physical rather than chemical action of the molecule[3]. Relatively weak physical forces are involved and these are frequently thought to be associated with the London dispersion force[4]. Not surprisingly there is good correlation between anaesthetic potency and molar refraction[5], polarizability of the anaesthetic agent[6], molal volume, solubility in olive oil[7–10], boiling points, and other properties of the molecules that are decided by the relatively weak long range interactions. Correlation of anaesthetic activity with the van der Waals a and b constants[11] is of particular interest here. The constant a is associated with the cohesive forces between molecules, and b with their volumes. The qualitative correlation of the van der Waals constants with anaesthetic potency has been discussed by Wulf and Featherstone[11]. Wilson *et al.*[12] demonstrated the correspondence between isonarcotic pressure of inert gases and increased hydrolysis of choline esters expressed as a function of $\sqrt{a}$.

Here we examine the correlation between anaesthetic potency of molecules and the van der Waals a constant in somewhat greater detail than has been done previously. We chose this parameter as it reflects the cohesiveness of the molecule and correlations with it presumably would reflect the interaction of the anaesthetic molecule with the receptor site. In Fig. 1 we plot the best value of the logarithm of the anaesthetic pressure producing loss of righting reflex in mice against $\sqrt{a}$. One of the difficulties in making such a plot is that no one laboratory has measured all the relative anaesthetic pressures; consequently, the various values used come from many sources. In Fig. 1 we use the values of Smith[13], who studied the pressures for the loss of righting reflex in mice reported by many sources and arrived at a "best" value. The plot in Fig. 1 is interesting because if He is included it gives a linear relation to within experimental error between log P_{an} versus $\sqrt{a}$ for about five orders of magnitude. We have omitted strongly hydrogen bonding anaesthetics and completely fluorinated compounds (although SF_6 is included to illustrate its marked deviation from the linear relation) and limited ourselves to agents that may be expected to form near ideal solutions.

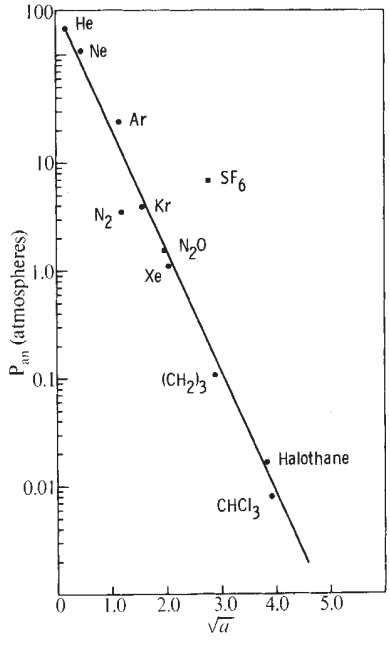

Fig. 1 Isonarcotic pressure in atmospheres plotted against the square root of the van der Waals a constant for various anaesthetics.

In a molecule such as SF_6 there is a large difference in the electronegativity of the fluorine and the central atom resulting in an excess of negative charge on the periphery. If the receptor site carries a negative charge, it would attract the SF_6 molecule with an affinity less than one would expect from the value of the molecule's van der Waals constant a. It is surprising that such a complex system appears to be described by a relatively simple function and this might be because there are compensating parameters involved.

We tested a working model of the anaesthetic-receptor system to see if it could provide us with a rationale of the correlation between log P_{an} and $\sqrt{a}$ as follows. When a nerve is excited, there is a large transient increase in Na^+ permeability of the fibre membrane, followed by a large transient increase of K^+ permeability. It is believed that the flow of ions takes place through aqueous channels[3] in the membrane which are opened or closed by an uncertain mechanism. When an anaesthetic dissolves in the membrane it expands which may reduce the size of any aqueous channels and so impede the flow of ions that anaesthesia occurs.

When anaesthetic molecules distribute themselves between the aqueous extracellular region and the membrane, the free energy change can be represented by

$$\Delta F = RT \ln \frac{C_1}{C_2} \quad \text{or} \quad \frac{C_1}{C_2} = \exp \frac{\Delta F}{(RT)} \tag{1}$$

where C_1 and C_2 are the anaesthetic concentration in the membrane and in the solution respectively, ΔF is the free energy change associated with the process and R is the gas constant and T the absolute temperature. When C_1 reaches a certain critical value, we assume that anaesthesia results. We will associate the dispersion energy with the free energy change in equation (1). It has been shown by London and others[14] that two neutral molecules interact with each other and this electronic dispersion energy is given by

$$W = -\frac{3}{2}\frac{\alpha_1\alpha_2}{r^6}\frac{E_1E_2}{(E_1+E_2)} \tag{2}$$

or for identical molecules

$$W = -\frac{3}{4}\frac{\alpha^2 E}{r^6}$$

α_1 and α_2 are the electric polarizabilities of the two molecules, r is the distances between their centres and E_1 and E_2 are their effective electronic excitation energies. Slater and Kirkwood[15] pointed out that the latter expression should be multiplied by $\sqrt{s}$ where s is the number of coupled oscillators contributing to the interaction.

Next we establish a relation between the van der Waals a constant and the polarizability α, by comparing the virial equation of state of a gas with the expanded van der Waals equation giving the following expression for the second virial coefficient

$$K_2 = b - \frac{a}{NKT} \tag{3}$$

Now when the interaction energy of a pair of molecules at a distance r apart is $-B/r^6$ when $r > r_0$ and infinite when $r < r_0$ the second virial coefficient can be shown to be

$$K_2 = \frac{2\pi N {r_0}^3}{3}\left(1 - \frac{B}{kT{r_0}^6}\right) \tag{4}$$

By comparing the two expressions for K_2 and incorporating the dispersion energy for two like molecules from (2) we get the following expression for a

$$a = \frac{1}{2}\frac{\pi N^2}{{r_0}^3} E\alpha^2\sqrt{s} \tag{5}$$

If this relation is substituted into (2) and then the result incorporated into (1) one gets

$$C_1 = C_2 \exp -\left[\frac{3}{2}\frac{(a)^{\frac{1}{2}}}{RT}\frac{\alpha_2}{r^6}\left(\frac{E_1E_2}{E_1+E_2}\right)\left(\frac{2{r_0}^3}{\pi N^2 E_1\sqrt{s}}\right)^{\frac{1}{2}}\right] \tag{6}$$

We do not imply that this equation gives an accurate distribution of the anaesthetic molecules between phases, but it is hoped that it does give a qualitative indication of the parameters that decide this distribution. It is clear from this equation that one should expect a linear log C_1 against $\sqrt{a}$ dependence if the other terms in the exponential expression are approximately constant. Part of this approximate constancy is compensatory. For example, as an anaesthetic molecule increases in size, the term r_0 in the numerator of the exponential factor increases but as the $\sqrt{s}$ in the denominator also tends to increase there is a tendency to compensate this. In addition, the excitation energies do not change greatly as one goes from one anaesthetic molecule to another. The strongest dependence in the exponential factor is the r^{-6} term. A possible rationale for the apparent constancy of this term is that in essence r is decided by the size of the receptor site (either an aqueous channel or a cavity formed by a carrier molecule). A small atom like He residing in a membrane site will have the same r value as a larger atom such as xenon since the distance from the centre of the atom to interacting points will be about the same in both cases. Finally, one should note the temperature dependence; and in this connexion, it is interesting to note that when the squid giant axon is lightly narcotized with ethanol, the height of the action potential is reduced and that this reduction can be restored by cooling[16].

The work of Johnson and Bangham[17] on the temperature dependence of the effect of anaesthetic agents on K^+ permeability in a model membrane and the studies of Johnson and Miller[18] on the antagonism of pressure in anaesthesia are also compatible with conclusions reached in this work. We conclude, therefore, that the correlation of log of anaesthetic pressure against $\sqrt{a}$ has a sound thermodynamic basis and may express a general principle of narcosis by chemically inert materials.

This study was supported in part by the Office of Naval Research and by an Ayerst Laboratories grant-in-aid.

We thank Professor D. Benson for helpful discussions and encouragement.

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Received September 18; revised November 20, 1972.

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BOOK REVIEWS

The Sixteen Faces of Leo Szilard

The Collected Works of Leo Szilard. Scientific Papers. Edited by Bernard T. Feld and Gertrud Weiss Szilard. Pp. xxii+737. (MIT: London and Cambridge, Massachusetts, September 1972.) $17.50.

THE dust cover of the first volume of the *Collected Works of Leo Szilard* shows sixteen photographs of Szilard, each presenting a different facial expression. These sixteen faces could match the different facets of Szilard's activities. In this age of high specialization, when any one scientist can push forward the frontiers of knowledge only in a tiny sector, it is extremely rare to find a person who encompassed and moulded such a wide range of subjects. But then Szilard would have been a remarkable personality at any period of time. His fields of interest were physics, biology, sociology and technology, and to each of these he brought in refreshingly new concepts and a highly original approach. He was an inventor and an innovator, a path-finder and prognosticator, but he did not create a school, because he was too much of an individualist and a nonconformist. He was always ahead of time and, therefore, usually out of phase with his contemporaries. By the time his ideas became accepted he had already forged ahead to take up a new attitude. Szilard himself illustrated this characteristic in a story which he liked to tell about himself. He served once on a jury in a murder case. When the first vote was taken, eleven jurors were in favour of convicting, but one—Szilard—was for acquittal. Since unanimity was required, the discussion was resumed and Szilard expounded his arguments. After some time another vote was taken; the result was again eleven to one, but this time eleven were for acquittal, and one for conviction: the odd vote was Szilard's who had in the meantime changed his mind!

This nonconformity is partially responsible for the f ctathat for a man of his tremendous achievements and influence, he had remarkably few publications; he published less than thirty papers in scientific journals. However, he had written a number of reports for the Manhattan Project (war-time work on the atom bomb), articles, memoranda and letters which contain many original ideas, both scientifically and sociologically; much of this material is of immeasurable value to the historian of science and current affairs. It was, therefore, an admirable and public spirited act by Professor B. T. Feld (who collaborated with Szilard in science and in world affairs) and Dr Gertrud Weiss Szilard (Leo's wife) to undertake the task of collecting and editing all his work. The first volume which has been published so far contains Szilard's scientific contributions. Apart from reprints of papers which appeared in various journals, it contains in full the reports relating to the Manhattan Project and much other material published for the first time.

It was an ingenious idea of the editors to divide the volume into several sections, each dealing with a specific area (thermodynamics, nuclear physics, Manhattan Project, biology, patents) and to precede each section with an introduction by a person who collaborated with Szilard in that particular area. These introductions, together with the foreword by Jacques Monod, the preface by the editors, and the *curriculum vitae* written by Szilard himself, make it much easier for the reader to understand Szilard's personality and to evaluate the importance of his contributions. The proper juxtaposition of published papers with private letters and declassified reports helps to fill in gaps, and to emphasize the role played by Szilard in one of the most notable periods in science.

Szilard was primarily a theoretician but his engineering upbringing enabled him to devise experiments which always worked. He had an uncanny instinct for the essential elements in a given field, be it nuclear physics, technology, biology or politics, and concentrated his efforts on those. Although he never headed a department of his own, he always managed to induce people to collaborate with him. For most of his life he was a knight errant, wandering from laboratory to laboratory, and leaving his mark in each place of halt. In 1934 he paid a short visit to St Bartholomew's Hospital in London, and in the course of a few months made several important discoveries, including the Szilard–Chalmers reaction, which put the Physics Department at Bart's, perhaps the smallest at that time, on the world map of nuclear physics.

His most important role was probably in relation to the release of nuclear energy. His own account makes fascinating reading. In 1933, soon after the discovery of the neutron, Rutherford was reported to have said at a meeting of the British Association that talk of large scale liberation of atomic energy was moonshine. This piqued Szilard, who felt that neutrons, which do not ionize and, therefore, must interact with nuclei, could be used to produce a chain reaction in which the capture of one neutron would give rise to the emission of further neutrons. Not knowing which nucleus would be most suitable, he wanted to carry out a systematic survey of all ninety-two elements; the cost of this project was estimated to be $8,000. However, the funds did not materialize and the survey did not get under way. It was not until 1939, after the discovery of fission, that he observed —at the same time as Joliot—the emission of neutrons, thus opening the way to a practical chain reaction. He followed this up very quickly with the design of a nuclear reactor for which he filed and was later granted a patent.

Speculating what would have happened if fission of uranium were discovered—as it should have been—in 1934, and considering the political situation in Germany at that time, Szilard concluded that those who missed this discovery should have been awarded the Nobel Prize for Peace! Somehow, the Nobel Prize—whether for Physics or for Peace—eluded Szilard, but posthumously he achieved another unique distinction: a crater was named after him on the far side of the Moon, an appropriate memento to the man

who dared to defy Rutherford in showing that nuclear energy was not "moonshine". Actually, the naming of a crater after him was predicted by Szilard in his book, *The Voice of the Dolphins*, in connexion with his political activities, but this is another story, which will be told in the second volume of the *Collected Works of Leo Szilard*. Its publication is eagerly awaited.

J. ROTBLAT

Neurotransmission

Perspectives in Neuropharmacology. Edited by Solomon H. Snyder. (A Tribute to Julius Axelrod.) Pp. xi+404. (Oxford University: London and New York, September 1972.) £7.75.

THIS book contains twelve articles written by former colleagues of Dr Julius Axelrod, to whom the book is dedicated. The articles give a picture of the main direction of research into chemical transmission in the brain, and into the biochemical processes involved in transmitter metabolism. The articles form an interesting and valuable collection.

L. T. Potter and P. B. Molinoff describe the isolation of cholinergic receptor proteins. A receptor antagonist resembling a choline ester (benzilylcholine mustard) has been synthesized by Gill and Rang. This is an irreversible blocking agent of muscarinic receptors. Potter and Molinoff find that alpha-bungarotoxin, obtained from the venom of a snake common in Taiwan, is a specific labelling agent for nicotinic receptors. The toxin blocks all responses to acetylcholine (ACh) in electric tissues (of *Torpedo*) and skeletal muscles, without affecting resting or action potentials, the release of ACh, or acetylcholinesterase. A comment may be made on the authors' use of the term "cholinergic". Dale used the term to distinguish sympathetic fibres which "worked" through the release of ACh (like those to the sweat glands) from those which "worked" through the release of something like adrenaline; these were adrenergic. Hence "cholinergic" can be applied to nerves, but not to receptor proteins.

S. H. Snyder and K. M. Taylor deal with histamine in the brain, and discuss whether it is a transmitter. Histamine is synthesized maximally in the hypothalamus. It appears to be localized to nerve terminals in the adult rat brain and to be contained in synaptic vesicles. But histamine is peculiar in occurring in higher concentration in the newborn rat brain than in the adult. There is no evidence that there is an uptake of histamine by nerve terminals as there is of noradrenaline.

L. L. Iversen has written on the uptake, storage, release and metabolism of GABA in inhibitory nerves. This article is full of interest. Gamma-aminobutyric acid is now known to be released by the inhibitory neuromuscular nerves of crustaceans, and also by the inhibitory fibres from the Purkinje cells of the mammalian cerebellum which run to Deiter's nucleus. The inhibitory action is due to the transmitter stabilizing the resting membrane potential of the postsynaptic cell, and GABA does this by causing a selective increase in the permeability to chloride ions, thus stabilizing the membrane near the chloride equilibrium potential. A second way in which the inhibitory transmitter may act is at synapses on presynaptic excitatory terminals to reduce the amount of excitatory transmitter released. GABA mimics very closely the action of the naturally occurring inhibitory transmitter; it is present in high concentrations in inhibitory motor neurones, but not in excitatory neurones. The enzyme which synthesizes GABA (glutamate decarboxylase) is present in inhibitory neurones, but not in motor neurones. The alkaloid bicuculline blocks the actions in the mammalian brain, and also the naturally occurring inhibitory transmitter at neurones (insensitive to strychnine) in the cerebral cortex, the cerebellum and the hippocampus. GABA-inhibitory neurones have a specific GABA-uptake mechanism, and mammalian brain slices accumulate exogenous GABA. Release of GABA by the stimulation of the inhibitory motor nerve has been demonstrated in the lobster nerve-muscle preparation. The amount released is proportional to the frequency of stimulation.

R. J. Wurtman and J. D. Fernstrom write on the control of brain monoamine synthesis. They have found that physiological changes in plasma tryptophan influence the amount of serotonin in the brain. Thus administration of a low dose of 12.5 mg kg^{-1} tryptophan to rats at a time when plasma and brain concentrations are lowest causes an increase in brain serotonin of 20–25%. The administration of doses of insulin (1–2 U kg^{-1}) which cause a rise in plasma tryptophan also cause a rise in brain tryptophan and serotonin. A carbohydrate meal eaten by a rat also causes a rise in plasma tryptophan, brain tryptophan and brain serotonin. This may be due to the secretion of insulin caused by the meal.

H. Thoenen writes on chemical sympathectomy by 6-hydroxydopamine, an amine which is taken up by adrenergic neurones and in large doses causes degeneration of the terminals. In the newborn animal it causes degeneration of the ganglion cell bodies. In the adult cat nictitating membrane this degeneration lasts for about 14 weeks. When a large dose is given to a cat, and the heart is then perfused, there is a long lasting discharge of noradrenaline. This discharge does not occur in the absence of calcium. Evidence that 6-hydroxydopamine must first be taken up is provided by the fact that its action is blocked by desmethylimipramine which prevents the uptake of noradrenaline. Thoenen says that the changes produced by 6-hydroxydopamine do not affect the Schwann cells or cholinergic nerve endings. However, he does not say whether the narrow band of acetylcholinesterase, which Eränkö showed closely investing the adrenergic terminals in the rat pineal, is affected.

J. H. BURN

Logic Design Techniques

Logical Design of Digital Circuits. By C. M. Reeves. Pp. v+192. (Cambridge University: London, November 1972.) £1.60.

Logic Design Algorithms. By D. Zissos. Pp. x+458. (Oxford University: London, November 1972.) £9.

THERE are already so many books published on logic design techniques that the appearance of yet more texts must be viewed with some apprehension; however, these new books appear to offer a somewhat different approach to the subject.

Logical Design of Digital Circuits is intended primarily for computer science students and, as such, adopts a formal mathematical approach to the subject, drawing heavily on Boolean algebra. In many cases this over-complicates the subject, especially for engineers, and a more physical interpretation would have been preferable. For example, the solution of simultaneous Boolean equations is excellently dealt with and is given a fairly detailed treatment. However, though in theory simultaneous equations are important, particularly in the design of sequential circuits, in practice they are seldom, if ever, used.

It is very encouraging in this book to see software techniques being considered as an integral and alternative aspect of logical circuit design; the two disciplines have been separated for far too long. Further progress in the design of logic systems depends very much on the realization and acceptance of the duality of hardware and software implementation. In particular the use of Backus–Naur Form for defining binary sequences, as for example in describing the operation of finite-state machines, is to be applauded. It is interesting to note in this respect the similarity of BNF to the regular expression method of defining sequential machines. Another useful feature of the book is the emphasis on logic simulation, and the inclusion of an ALGOL listing for a simple simulator package called SOLD.

Overall the book is fairly comprehensive, including chapters on Boolean algebra, the design of combinational circuits, and clocked sequential networks, and concludes with a chapter on computer circuits; the subject of asynchronous logic is omitted entirely. There are very few errors in the book, but there are a number of misleading assumptions: for example, relating the cost of a circuit directly to the number of inputs. In addition the logic symbols used could have been better specified; for instance, bistables are shown without a clock input: this can be misleading, as in the case of the circuit of Fig. 2–11, which would malfunction unless the bistable was clocked. The book contains numerous problems, some of which have worked solutions; a short bibliography is given at the end of the text. In my view the book is good value and could certainly be recommended (with some provisos) as an introductory text for computer science students.

Logic Design Algorithms is primarily intended to be a handbook of design techniques for the professional engineer, and as such it adequately fulfils its function. The text gives detailed algorithms (fourteen all told) for the design of combinational, asynchronous and synchronous logic, using NOR/NAND gates and bistables for the implementation. The book, however, has a rather curious approach to the subject and gives the impression that the work, though highly original, has been developed independently of established switching theory. This has the disadvantage that it would be difficult for the non-specialist to relate the terminology used in the text to existing work. For example, in the discussion of asynchronous logic no mention is made of the usual circuit classification into fundamental mode, normal mode, pulse mode, and so on.

The algorithms themselves, based as they are on algebraic techniques, seem rather too complex for hand computation. It is intended, however, that the algorithms should eventually be implemented on a digital computer for automatic computation. The design methods used achieve good results, particularly in the case of combinational circuits which allow non-canonical input terms and take account of hazard-free and multi-level implementation. Unfortunately, there are a number of important points which require further clarification. For example, in the discussion of circuit hazards no mention is made of dynamic hazards due to unequal signal paths; it would appear that the algorithms do not allow for this condition.

The techniques described for sequential circuit design do not seem to offer any great advantages over existing methods. The derivation of turn-on and turn-off sets, and their subsequent reduction, is directly analogous to the usual practice of extracting bistable input equations. Moreover, the algorithms can be at fault when dealing with don't-care conditions. For instance, in Fig. 4.46b B and $\overline{B}$ both go to logic I for the input condition $\overline{A}$ B K C, indicating that the SR bistable input constraint of SR=0 has been violated.

The book contains a copious number of worked examples in the text and an appendix of specimen test papers; very little reference is made to current work in the field, and there is no bibliography. Though it is suggested that the book would be suitable for student use, in my opinion this is not so, due to the unusual treatment of the subject.

D. W. LEWIN

Propaganda and Eugenics

Genetics and American Society. By K. M. Ludmerer. Pp. xi+222. (The Johns Hopkins Press: Baltimore, Maryland, 1972.) $10.

THOSE historians and theorists intent on dismissing the "use–abuse" model of the interaction of science with society would do well to read this book carefully. They may be hard pressed to suggest an alternative explanation of the events described. Though "genetic science is intimately related to society", Mr Ludmerer has unravelled enough threads in the past fabric of that science to show that its relations are not necessarily ideological; that most genetic research is and has been intellectually independent of the old-style eugenic social activism which "abused" its findings.

Eugenists and their supporters, from the early years of this century until the 1930s, claimed a scientific base for their often prejudiced political and social goals. Most human geneticists saw very early that their theories were being misused and ceased to support eugenic proposals. That the eugenists were, in America, successful in putting through state sterilization laws and the Federal Immigration Restriction Act of 1924 was a function of their political strength rather than their scientific support. Geneticists of the time despised eugenics, but did not have effective institutional bases from which to oppose eugenic propaganda. Indeed, eugenic abuses had a devastating effect on the advance (measured in terms of public support, financial endowment, publishing outlets, and so on) of genetic science itself. After the Second World War, however, longevity and the atomic bomb brought new ("clean") medical and scientific interest in human population and radiation genetics. At the same time, American revulsion at Nazi eugenic actions killed the old movement, and left new, rather less strident and more theoretical, eugenics groups. The present-day eugenics picture contrasts strongly with the racialist, authoritarian and simple-minded view of inter-war eugenists; and human genetics has, of course, become thoroughly respectable.

This is the story Mr Ludmerer tells, in a fairly well-written fashion, although he is a little repetitive and fond of quotations. His published and archival documentation is thoroughly scholarly, and primary material is usually interpreted with care. But Mr Ludmerer's recent interviews with the scientist–actors of his story constitute secondary sources, which he too often takes at face value. This is understandable in view of the libel laws, but it does lead to some white-washing of the "everybody-else-was-a-racist-but-not-me" variety.

Finally, there are three small interpretive "mistakes" in the book: (1) Mr Ludmerer underrates the quality of membership in the early British Eugenics Education Society, and the influence of British eugenists generally; (2) he has an unhealthy negative attitude towards amateur scientific activity; (3) he does not recognize the very complicated way in which "biologism" in sociology retained and expanded its influence, while seemingly losing it.

But these minor faults do not affect the importance of the book as perhaps the best history to date of the social aspects of American genetics and eugenics. It is a valuable contribution to our understanding of a subject about which we must remain politically, socially and intellectually sensitive.

JAMES FRIDAY

Mechanical Design

The Selection of Design. By Gordon L. Glegg. Pp. 84. (Cambridge University: London, November 1972.) £1.85; $6.95.

IN a first approach to design there is a tendency to divide it into three areas: visual design, mechanical or structural design, and the rest. It is in this sequence that design is appreciated popularly and, as we may note, by politicians. At the national level the official stimulation of design has now passed from the Council of Industrial Design (visual mode) to its transformation the Design Council (visual plus mechanical mode).

Interesting and valuable aspects of design such as involve electronic circuits, computer software, chemical processes, and so on, lie within the *terra incognita* of the third area. Ongoing arguments suggest that architects are uncertain about the areas to which they owe allegiance.

After this preamble it is possible to

state that Glegg's book uses "design" in the sense of the second area. This is implicitly the only place where design takes place. It is a rather boring place where "there is no unsophisticated engineering left, or there shouldn't be". What this means is that most of the basic devices or ways of doing things have already been invented or discovered. From this notion derives the term "selection" in the title of the book. The activity of design is largely concerned with the selection of possible ways of doing things from an apparently existing catalogue. From the many possibilities one eliminates by selection by context and by selection of content. Somehow economics takes a back seat. Very occasionally there are apparent impossibilities to tackle. This is the creative activity.

Within his limits Glegg has produced a very interesting little book which is essentially of the "how I" rather than the "how to" kind. Whether it will be suitable to young inventors will depend upon their lines of activity. In some ways they may have to be interested in the bits and pieces of motor cars. But there is no suggestion of further reading or hints of what other people have been doing in the design business and how his ideas compare with theirs.

SYDNEY GREGORY

Photosynthetic Enzymes

Methods in Enzymology, Photosynthesis and Nitrogen Fixation. Edited by Anthony San Pietro. Volume 24, Part B. Pp. vii+526. (Academic: London and New York, September 1972.) $23.50.

AN up-to-date method book for photosynthesis research has been needed for a long time. The present book fills this need by giving an almost full account of a vast number of experimental approaches, ranging from biochemical to physical, to this multi-faceted subject.

Of the two volumes dealing with photosynthesis and nitrogen fixation, part A has been devoted to preparatory methods. Part B, which is the matter of this review, is less homogenous; its main body is devoted to processes and measurements, but it includes preparative aspects as well in the sections on "Synthesizing Capabilities" and "Nitrogen Fixation".

The book is divided into four sections, containing forty-four chapters, each dealing with a different methodological aspect and written by a different author. Although this guarantees, in general, authority and a high standard of presentation, the book as a whole suffers from a lack of homogeneity and coherence. This could be perhaps eliminated by elaborate editing and planning. The various chapters should have been grouped together according to being physical, chemical or biochemical, and further subgrouped into various sub-categories. For example, optical and photophysical methods could be grouped together, with an appropriate unifying introduction. The same is true with other categories. The division to small chapters also causes unnecessary repetition. For example, light intensity measurements are included in the chapters "Quantum Yields" and "Light Intensity Measurements". There are other examples of this.

The level and depth of the presentation are not equal. Besides very comprehensive treatises (to mention but a few: "Flash Kinetic Spectrophotometry", "Light Induced Paramagnetism", "Measurement of Hill Reaction") there are some small chapters that miss essential details. For example, methods of treating data in order to obtain the limiting quantum yield are absent; the chapter "Enhancement", besides defining the subject, gives just little more than how to measure a Hill reaction, and particular aspects regarding how to treat the data and methods to deal with non-linearity of rate with intensity (for example, the Kok effect) are not expanded; the small note on "A Green Safelight" could be expanded and included in the chapter on "Light Sources and Measurements"; the coverage of fluorescence and delayed light methods is too brief; the details of measuring O_2 evolution from single flashes, and the detection of other redox reaction by the oxygen electrode, although mentioned, are also too brief.

There are descriptions of very specific methods, which have not been extensively and independently reviewed. For example, the method of high derivative absorption spectrum to separate peaks in a complicated spectrum should be applied with caution (since each derivative adds, in principle, an additional peak to the original spectrum) and probably is valid only under limited conditions. Also, the method of steady-state relaxation spectrophotometry, although a very general tool in chemical kinetics, seems to have only a limited application here.

The coverage of the typical biochemical and preparative chapters seems adequate. In fact, the writing of a typical biochemical method involves far fewer problems and is more or less standardized compared to the writing of a physical method.

These drawbacks, serious as they are, do not impair the usefulness of the book, which serves as a quick reference and source for most of the methods existing in the field, and is thus very welcome.

S. MALKIN

Extinction by Hormone

Insect Juvenile Hormones: Chemistry and Action. Edited by Julius J. Menn and Morton Beroza. (Proceedings of a Symposium held in Washington, September 1971.) Pp. xv+341. (Academic: New York and London, June 1972.) n.p.

Insect Sex Pheromones. By Martin Jacobson. Pp. xii+382. (Academic: New York and London, October 1972.) $22.50.

JUVENILE hormone functions during insect metamorphosis by actively favouring the differentiation of larval characters, and in many species it also controls oocyte vitellogenesis in the adult female. If the corpora allata—the glands which produce juvenile hormone—are removed from early larval instars, dwarf adults can result, and if the hormone is introduced when it is normally absent, supernumerary larval instars or monsters intermediate between larva, pupa and adult can be produced. Moreover, when juvenile hormone is applied to the eggs of some insects, embryogenesis can be abnormal or post-embryonic development can be affected. It is not surprising, therefore, that the prediction was made that juvenile hormone could prove to be a potent insecticide—with the advantages over current synthetic insecticides that, being a natural compound, it would be unlikely to have adverse environmental effects, and that the insects would be unlikely to develop immunity to one of their own hormones.

Juvenile hormone was discovered nearly forty years ago, and its chemical identity was established in 1967 as methyl 10,11-epoxy-7-ethyl-3,11-dimethyl-2-6-tridecadienoate. A second hormone, with a methyl group replacing the ethyl at C_7, was described a short time later. In the years immediately before the identification of juvenile hormone, other chemicals were discovered which mimicked its activities, and families of compounds are now known which have greater or less juvenile hormone activity in a variety of insect species. These discoveries raised the possibility that hormonomimetic chemicals could be tailored for use against specific insect pests.

Insect Juvenile Hormones discusses these aspects of juvenile hormone and its mimics in the light of recent evidence. The volume contains contributions on the chemistry, biochemistry, metabolism and action of the authentic hormones, and of many hormonomimetic chemicals. Preliminary field trials of some of the compounds are described, together with tests on their environmental stability and decomposition products. Very brief details of their toxicity to mammals and plants are also provided.

The papers are reproduced by a photographic process to hasten publication and the consequent differences in style and format are sometimes disconcerting, especially when the text appears to run on into the legends of tables and figures.

Overall, the impression gained from this volume is one of muted optimism. Professor Schneiderman points out that young larvae seem to be quite insensitive to the compounds, which is unfortunate since these stages often cause most damage. Moreover, insects can inactivate, sequester or excrete juvenile hormone at certain stages in their development, so the hope of not developing immunity to exogenous hormone is unlikely to be fulfilled, even more so with foreign chemicals. The results of the field trials are disappointing, although some compounds show promise in the control of certain insect pests. The hormones and their mimics operate during critically short developmental periods, so that application must be precisely timed or the compounds must persist for adequate periods. Results described here suggest that field stability is low. The compounds do not have the dramatic knockdown properties of current insecticides and the effects of application may not be manifest until the next generation. Their use as ovicides seems the most encouraging, for they can be applied to the adult female or her freshly laid eggs, and if embryogenesis is not interrupted, metamorphosis may subsequently be prevented. Considerably more needs to be known of the detailed developmental life-histories of insect pests and the ways in which juvenile hormone and its mimics control and affect morphogenesis, reproduction and behaviour before the hope of specifically tailored insect pesticides becomes a reality. But these "third generation insecticides" will undoubtedly play a major part in any programme of integrated control of insect pests.

Insect Sex Pheromones also contains a chapter on natural chemicals in insect pest control: sex attractants can be used to lure members of the opposite sex in large numbers to particular sites. Or the atmosphere can be saturated with the attractant, causing confusion and disruption of the normal orientation mechanisms so that mating is prevented. This form of control suffers from one of the disadvantages of the juvenile hormone mimics—that the damaging young stages are unaffected and the agriculturalist must look beyond the loss of his current infected crop to subsequent pest-free growth. The author has collected an enormous amount of factual information on the occurrence, identification and synthesis of insect sex attractants, but presents it in this volume in a catalogue style with little attempt at synthesis or the development of concepts and theories. With 1,337 publications listed, the book will be invaluable as a reference source.

Both *Insect Juvenile Hormones* and *Insect Sex Pheromones* illustrate the results of fruitful marriages between chemistry and biology, which should engage the hopeful interest of all those concerned with the environmental problems of current insecticide pollution.

K. C. HIGHNAM

Carbohydrates

Stereochemistry of Carbohydrates. By J. F. Stoddart. Pp. xi+249. (Wiley Interscience: New York and London, 1971.)

ONLY infrequently is a new textbook sufficiently unusual to have no near competitor. Dr J. F. Stoddart has written such a book and in doing so has presented carbohydrate chemistry in a new light, and moreover in a way designed to attract the attention generally of organic chemists. In the past there has been a tendency to treat carbohydrate chemistry as an isolated aspect of organic chemistry, rather than as part of aliphatic and heterocyclic chemistry. However, many of the problems which arise in carbohydrate chemistry are stereochemical in nature and answers are to be found in a knowledge of the structural and dynamic aspects of stereochemistry. In this book there is a thorough but concise account of carbohydrate chemistry in stereochemical language. The important role of isomerism in carbohydrate chemistry is high-lighted and the account serves to illustrate the almost unique status amongst organic compounds of the sugars, because constitutional, configurational and conformational isomerisms are often superimposed on each other in this group of natural products. The author has achieved a happy union of stereochemistry and carbohydrate chemistry.

The book has five chapters, each concluded by a list of references, and an author and subject index. In the first two chapters basic principles are presented and so to use the book little previous knowledge of modern stereochemical concepts is required. Definitions are clearly stated. Other chapters are devoted to the constitutional, configurational and conformational isomerism of sugars; the interplay of these forms is explored. To avoid confusion arising from the specialized and often bewildering nomenclature of carbohydrates, many illustrations are provided, to the extent that a formula is given for almost every compound mentioned. Discussion of the stereochemistry of polysaccharides has been integrated with that of simpler carbohydrate derivatives.

The physical methods which are particularly suited to providing answers to stereochemical problems posed by carbohydrate molecules are surveyed. These include X-ray diffraction, mass spectrometry, infrared and nuclear magnetic resonance spectroscopy, dipole moments and optical rotations. This coverage is commendable because in the investigation of stereochemical problems it is desirable to employ as many physical techniques as possible.

The book is excellent for its description of the systematic application of conformational principles. It is surprising, and even unfortunate, that those concerned with conformational analysis have not drawn extensively on carbohydrates to exemplify conformational principles. It is not always appreciated that carbohydrate chemistry has made important contributions to conformational theory. For example, the term "conformation" was first introduced by Haworth in 1929 in connexion with the shapes of sugar molecules, and cellulose is probably the first molecule in which the chair shape of the six-membered ring was detected through X-ray analysis. The effect of conformation on reaction rate was probably first recognized in carbohydrates by Isbell in 1937, and Reeves's discussion in 1949 of the conformations of sugars antedates by a year Barton's pioneering paper on conformational analysis. The first major recognition of the importance of conformation in n.m.r. spectroscopy is found in a paper in 1958 by Lemieux, Bernstein and co-workers. The author's readable account of the facts (both qualitative and quantitative) of carbohydrate conformations, and his discussion of concepts, should stimulate investigators in other branches of organic chemistry to turn to carbohydrates as suitable, and often readily available, models to illustrate or confirm stereochemical principles.

The book will be of interest also to graduate students and research workers in organic stereochemistry and natural product chemistry, as well as to those specializing in carbohydrate chemistry and conformational analysis. Teachers of degree courses in chemistry will find valuable reference material. Extensive use of the book over a period of months has shown it to be both reliable and informative. The selection of topics for discussion in the book is necessarily personal to the author, but is sufficiently wide to achieve adequately the aim of bridging the gap between carbohydrate chemistry and stereochemistry. This is a textbook which was badly needed. The author is to be congratulated on his efforts and his book is strongly recommended.

W. G. OVEREND

Nature's Allograft

Nature's Transplant. The Transplantation Immunology of Viviparity. By J. Maxwell Anderson. Pp. viii + 145. (Butterworth: London, November 1972.) £3.

A MAJOR unsolved problem in transplantation biology is how the mammalian embryo manages to survive in the uterine environment of a genetically differing female without eliciting the expected immunological rejection reactions. The embryo seems to be exempt from the "laws of transplantation immunity", and as such is of considerable interest not only for its own sake but for the possibility of providing valuable clues to the attainment of successful surgical grafts of tissues and organs and to an understanding of the cancer–host relationship.

The precise nature of the problem was defined in 1953 in an eloquent essay by Medawar[1], and most of the experiments subsequently carried out in attempts to elucidate this paradox of transplantation immunology have been within the philosophical framework of that assay. Maxwell Anderson's short book is essentially a contemporary assessment of the old problem. It deals comprehensively with various theories put forward by Medawar and others, and considers much evidence to support or undermine them. The embryo's survival is undoubtedly due to a number of biological adaptations, including the specialized nature of its vascularization, the selective placental "barrier" restricting any large-scale traffic of maternal immunologically competent cells, and the establishment of a complex foeto-maternal immunological equilibrium following exchange of antigenic information. The presence of blocking antibody is thought to be one of the factors involved in this equilibrium.

Despite the blurb's assertion that "This is the first unified account of the subject", Anderson's analysis is very much in line with many of the reviews that have appeared in the past four years, although his "Integrative Hypothesis" of chapter 7 does include one or two new thoughts. The book contains eight plates on embryology and tissue grafting in the armadillo, which might be thought excessive considering the wide scope of the text.

In general, it is difficult to see the market for this book. It is far too detailed for anyone not involved in research in the field (no fewer than seventy pages out of the total of 145 are taken up by reference lists), whilst those that are would probably find Beer and Billingham's recent review[2] of more value.

W. D. BILLINGTON

[1] Medawar, P. B., *Symp. Soc. Exp. Biol.*, **7**, 320 (1953).

[2] Beer, A. E., and Billingham, R. E., *Adv. Immunol.*, **14**, 1 (1971).

Solid State Spectroscopy

Optical Properties of Solids. By Frederick Wooten. Pp. xiii+260. (Academic: New York and London, October 1972.) $12.95.

SPECTROSCOPIC studies provide a wealth of information about the various excitations which occur in crystals, and in spite of the very large amount of research which has already been carried out, solid-state spectroscopy still throws up a steady flow of new phenomena. The book under review aims to supply a distillation of this active research field, with the fundamental principles of the subject described at a level suitable for first-year graduate students. The material is restricted to the optical properties associated with electronic transitions in perfect crystals. There is some discussion of surface effects in addition to the more familiar bulk properties of electronic excitations in the effectively-infinite crystal. The author is concerned throughout the book to explain the theoretical concepts behind the spectroscopic properties. He has taken pains to provide simple derivations of the main theoretical expressions, and it seems to me that his mixture of mathematical rigour and physical insight achieves a suitable balance for his intended readers. The theoretical results are illustrated throughout the book by a well-chosen selection of experimental data, but the emphasis of the work is theoretical, and there is no attempt to provide a survey of experimental results or techniques.

The main topic of the book is the interaction of electromagnetic radiation with electronic transitions. The quantum mechanics of transition rates, absorption and dispersion of radiation is presented in some detail. The dielectric function is used throughout as a bridge between the microscopic electronic properties of solids and the macroscopic concepts used in the description of optical phenomena. The associated theoretical machinery of linear response functions and the fluctuation-dissipation theorem is covered, with particular reference to electronic spectroscopy. Typical spectra of metals, alloys and semiconductors are described and interpreted in terms of the theory. The techniques of photoemission and characteristic energy-loss spectroscopy are shown to be valuable for the complementary information they can provide on the electronic energy-band structure.

The book can be recommended to readers with a good basic knowledge of solid-state physics and quantum mechanics who need a more specialized introduction to electronic spectroscopy. The style of writing and the printing are very readable, and the book is well produced except for a number of misprints which include the complete omission of equation (3.75) from the review copy.

RODNEY LOUDON

Multivariate Statistics

Multivariate Statistical Analysis: a Bibliography. By T. W. Anderson, Somesh Das Gupta and George P. H. Styan. Pp. x+642. (Oliver and Boyd: Edinburgh, 1972.) £10.

THIS new bibliography is one of those immeasurably important pieces of professional housekeeping to which no review can ever do full justice. Recalling that the senior author, Anderson, is the doyen of statisticians in this field of analysis, it is interesting to note that the project started (in 1963) from the desire of the three authors to understand and increase their own knowledge of the subject. This leads to a systematic search of the literature and on to a massive cooperative organization involving a computer-produced listing and large-scale photography of typescript.

The first chapter covers the available books, a classified list of 213 titles together with an author index, available in 1970 or earlier. This is followed by a chapter on the structure of the 819 journals and other collections of papers used, and here the terminal date is 1966, although there are some items in the Addenda at the end of the volume. The third chapter contains the core bibliography of the (nearly) 6,100 papers arranged alphabetically by author with analysis by year, language, numbers of authors and papers. This, of course, is the computer-produced portion and is a fine example of its kind; the organization is of general bibliographic interest. The collection of papers is indexed in chapter 4 in accordance with a subject-matter code described in detail in chapter 5 where is also found a straightforward index to this subject-matter code.

The preface contains a clear statement of the conditions for complete or selective coverage of the various aspects of this immense field of statistical techniques. At once it illustrates how real boundaries are growing less distinct as well as the need to draw some boundaries or be selective if any usable work is to result.

The statistical profession's collection of works of this kind is steadily improving and the authors, together with everyone associated with ABOMSA (as it has been affectionately styled), merit warmest congratulations and thanks for their magnificent contribution.

W. R. BUCKLAND

CORRESPONDENCE

Keynesian or Galbraithian?

SIR,—Surely government spending on research is wise economic policy, not because it is Keynesian, as you suggest (*Nature*, **240**, 515; 1972), but because it is Galbraithian. A non-growth economy, such as Galbraith and others have described, will need a place for the able and energetic individuals who in the past have found their rewards as entrepreneurs. Research is as open-ended as business or industry: it can absorb all the enthusiasm of the most able person. But research uses few natural resources, causes little pollution, and seldom contributes in any substantial way to the growth of the economy.

Is it not better for society to pay a man to do research, rather than to pay him to produce and sell goods which society neither wants nor needs? Subsidies to academics and artists to keep their talents out of business and advertising should have at least as high a priority with the government as subsidies to farmers to use their land for one crop rather than another. If we start now to increase the number of research studentships, as well as the funds for research itself, able undergraduates and schoolchildren will tend to steer towards a career in research rather than in business, and there will be fewer frustrated managers in 20 years time.

A substantial increase in the number of people who are focusing a trained curiosity on all aspects of mankind and his world could lead to a new burst of understanding, of the kind last seen during the Renaissance. We can have no more noble aspiration than to try to discover who we are and where we are going. Academics should point out to governments not just that research can be useful in the short term, but also that non-utilitarian research can be valuable.

Yours faithfully,

D. A. MARVIN

298 *Lawrence Street,*
New Haven, Connecticut 06511

Taxonomy and Evolution

SIR,—Professor J. W. Fairbairn queries the significance of the idea of evolution in the advancement of taxonomy (*Nature*, **241**, 225; 1973). He makes very generalized accusations against taxonomists without producing any definite evidence.

I have spent the greater part of the past thirty years in intensive taxonomic study of some tropical families of plants on which previously recorded observations were defective, and taxonomic treatments correspondingly unsatisfactory. I assert that the idea of evolution has always been an essential element in my thoughts on the problems presented by these families. The evidence that organisms have reached their present condition through processes of evolution does not depend only on the taxonomic study of existing organisms; there is much other independent evidence. Evolution implies that there is a built-in natural classification for organisms; our problem is to find it. To regard the results of a taxonomic study as potential evidence of the history of evolution in a particular case is not arguing in a circle. Thought on the possible significance of such evidence often leads one to further observations which may throw further light on the subject. The problem is a dynamic one; to deny phylogenetic thinking is to ignore biological reality. I have attempted to express my ideas on this subject, with reference to some particular families of plants, in a paper entitled "Comparative Morphology, Taxonomy and Evolution" (*Phytomorphology*, **17**, 36; 1967) and refer interested readers to that statement.

Yours faithfully,

R. E. HOLTTUM

Royal Botanic Gardens,
Kew

Noah's Ark

SIR,—We were pleased to see that your correspondents, Harkins, Stenzel and Black (*Nature*, **241**, 226; 1973) have noticed that our paper on protein polymorphisms in man (Haigh and Maynard Smith, *Genet. Res.*, **19**, 73; 1972) lends some support to the biblical story of Noah's Ark. What your correspondents have not appreciated is that the biblical story provides the best direct test of Kimura's neutral mutation theory at present available. There are as usual some internal inconsistencies in the account, but it is reported in Genesis, 7, 2–3, that only one pair each of the unclean animals were admitted to the ark and seven pairs each of the clean animals. It follows that if Kimura is right there should be a greater degree of polymorphism in cows and antelopes than in pigs, camels and ossifrages.

Yours faithfully,

JOHN HAIGH
JOHN MAYNARD SMITH

Mathematics Division and School of Biological Sciences,
University of Sussex

Darwin and the Creator

SIR,—Surely it is silly of J. W. Fairbairn (*Nature*, **241**, 225; 1973) to "treat the Genesis account of creation with as much respect as that of the biologist". The fact that the biological accounts are varied and unsubstantiated does not in itself mean that any other account has therefore to be put on the same level. Hypotheses come not only as rivals but in rival forms: the various biological accounts of creation fall into one form, whereas religious accounts fall into another form. Quite apart from the more detailed questions of scientific modelling, how does the Book of Genesis stand in regard to the principle of falsification?

The words by Darwin, which J. W. Fairbairn quotes, include in themselves this contrast, for the concept of a Creator is utterly different from that of the "fixed law of gravity". If one assumes the former, why should one accept the latter, and *vice versa*? The two concepts spring from models of the universe which are incompatible.

It is as well to remember that when Darwin published the *Origin of Species* he did so with an anguished regard to the nature of the society in which he lived and worked. He himself was a firm agnostic and his use of the word Creator must be taken as poetical in the same way that any sensible person takes the Book of Genesis itself.

Yours faithfully,

CHRISTOPHER MACY

The Rationalist Press Association Limited,
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Obituary

Lord Rosenheim

LORD ROSENHEIM, KBE, MD, FRCP, FRS, Emeritus Professor of Medicine at University College Hospital, London, and President of the Royal College of Physicians from 1966 until March of this year, died on December 2, 1972, at the age of 64. From 1950 to 1971 he was Professor of Medicine at London University and Director of the Medical Unit at University College Hospital Medical School.

He was knighted in 1967 and became a life peer in 1970, being created Baron Rosenheim of Camden. He was appointed Chairman of the Medicines Commission in January 1972 and in May was made a Fellow of the Royal Society by special election.

Max Leonard Rosenheim was born on March 15, 1908, and educated at Shrewsbury School, St John's, Cambridge, and University College Hospital, where he graduated in medicine in 1932, taking the MRCP in 1934 and proceeding MD (winning the Raymond Horton-Smith Prize) in 1938. From 1941 to 1946 he served in the RAMC, first as a medical specialist and then as consulting physician to the Allied Land Forces in South-east Asia. It was this period of his life which gave him his love of travel and his fascination with far-off countries which were to bear fruit again later.

During his undergraduate days at Cambridge he was interested in the mechanism of action of ketogenic diets which were then used for the treatment of urinary tract infections. He soon realized that beta-hydroxybutyric acid was the key metabolite and the related but more stable substance, mandelic acid, was found to be effective in treating these infections. The impact of this discovery would have been much greater had it not been for the nearly simultaneous discovery of the sulphonamides, but mandelic acid is still in clinical use. Rosenheim was also extremely interested in hypertension and was one of the first to convince the medical profession that it really was treatable.

Although these scientific advances were noteworthy, his outstanding contributions to medicine were in the clinical and administrative fields. He had high and humanitarian standards of care for patients as individuals and also a social conscience which found expression in the publication in 1970, with Jessie Garrad, of *Social Aspects of Clinical Medicine* and the setting up of the Faculty of Community Medicine jointly by the three Royal Colleges of Physicians. After the war he became deeply concerned with the various boards of London University, particularly in relation to the medical curriculum and examinations and to the overseas colleges to which London University was in special relationship. As a result he became an expert committee man with his advice being much sought after. The ties abroad were strengthened by his Sims Travelling Professorship (1958) and by his work as adviser for the British Council (of which he was Chairman of the Medical Panel) which took him to Nigeria, Ceylon, the West Indies and India. On these tours he always visited out of the way places and he appeared to know everyone in the overseas universities.

It was during his Presidency of the Royal College of Physicians that he was able to exercise his qualities and his imagination to the full, and the College, in its new Lasdun home, rapidly became one of the foremost centres of postgraduate medical education. Max went everywhere, and while politically we were losing an empire, medically we were gaining one. He persuaded the three Royal Colleges of Physicians to drop multiple diplomatosis and to unite in a common Membership examination —MRCP(UK). The MCQ paper of Part I of this examination became outstandingly successful and applications to take it came in from Ceylon, Egypt, Ghana, Iran, Malaysia and the West Indies. Under him the College appeal was launched, which reached its target of £500,000, and it is still being used for a number of research and other College activities, the best known of which is the campaign on smoking and health. He also started the practice of holding College lectures in provincial centres.

Lord Rosenheim was unmarried, and in spite of his distinguished position he lived an unpretentious life and was held in the greatest affection by all who knew him. He had innumerable friends whom he used to delight with his amusing and stimulating conversation—not only on medical matters but on books, music and fishing, which were his second loves. For many years he lived with his mother, to whom he was very devoted and who died only recently. Although 1972 brought in a new era for him he seemed just as buoyant as ever and ready for new ventures, but it was not to be, and by his death British medicine is the poorer.

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